



SOIL ORGANIC MATTER DYNAMICS: INFLUENCE OF SOIL DISTURBANCE ON LABILE POOLS

THESIS

Submitted in partial fulfilment of the requirements

for the degree of Doctor of Philosophy in Ecology

at the University of Canterbury

by

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2014

Soil organic matter's value lies in its dynamic nature.

William Albrecht, 1938

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisors, Dr. Pete Millard of Landcare Research and Dr. Matthew Turnbull of the University of Canterbury, who have played a pivotal part in my PhD research. Matthew's energy and enthusiasm have always been very encouraging; and Pete's expertise and our inspiring discussions have all been extremely motivating and set a very high standard to aim for.

I am very grateful to Landcare Research for hosting me over the last three years. I also acknowledge UC Doctoral Scholarship. Operational research funding was provided by the New Zealand Ministry for Business, Innovation and Employment to Landcare Research.

I am indebted to everyone who gave me a helping hand along the way. Graeme Rogers provided his technical expertise with many tricks of many trades both in the lab and in the field. Ellen Cieraad generously offered her time for numerous discussions and provided essential guidance with statistical analyses. I am grateful to Mike Beare and Denis Curtin of Plant and Food for permission to work on the Millennium Tillage Trial and multiple conversations along the way. Discussions with David Whitehead, Andy Midwood, John Hunt, Scott Graham and Matti Barthel helped me shape my ideas. Michelle Peterson, Weiwen Qiu, Kathryn Stillwell, Craig Tregurtha, Goudong Yuan, Tony McSeveny, Anitra Fraser and Karen Boot provided their technical expertise and guidance at various stages of my PhD journey. Elena Molchanova provided statistical advice. Besides, I want to thank my running friends and flatmates, who have also contributed to my PhD experience.

Lastly, I thank my parents, Olga Zakharova and Igor Zakharov, and my grandparents, Irina Tamarina and Yuriy Tamarin, for being academic and sport models and providing ongoing support and encouragement, even from so far away. My partner, Rowan Hooper, also deserves a big thank-you: for his support, inspiration and encouragement.

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LIST OF ABBREVIATIONS

A	surface area
AICc	Akaike information criterion corrected for small sample sizes
a.s.l.	above sea level
C	carbon
$\delta^{13}\text{C}$	isotopic signature of solid material
$\delta^{13}\text{CO}_2$	isotopic signature of soil-respired CO_2
$\Delta^{13}\text{CO}_2$	standardised isotopic signature of soil-respired CO_2
DI	deionised water
DOC	dissolved organic carbon
E	evolution rate of soil-respired CO_2
FACE	free air CO_2 enrichment
HWEC	hot water extractable carbon
LogLik	maximised log-likelihood
MAT	mean annual temperature
MTT	Millennium Tillage Trial
POM	particulate organic matter
R	$^{13}\text{C}/^{12}\text{C}$ ratio
SOM	soil organic matter
SSA	soil surface area
t	duration of incubation
TDL	tunable diode laser
V-PDB	Vienna Pee Dee Belemnite standard
W_A	water content of air-dried soil samples
WSC	water soluble carbon
y_{start}	initial soil-respired $\delta^{13}\text{CO}_2$
y_0	equilibrium soil-respired $\delta^{13}\text{CO}_2$

ABSTRACT

Soils are the largest pool of carbon (C) in terrestrial ecosystems and store 1500 Gt of C in their soil organic matter (SOM). SOM is a dynamic, complex and heterogeneous mixture, which influences soil quality through a wide range of soil properties. Labile SOM comprises a small fraction of total SOM (approximately 5%), but due to its rapid turnover has been suggested to be most vulnerable to loss following soil disturbance. This research was undertaken to examine the consequences of soil disturbance on labile SOM, its availability and protection in soils using the isotopic analysis of soil-respired CO₂ ($\delta^{13}\text{CO}_2$).

A range of soils were incubated in both the short- (minutes) and long-term (months) to assess changes in labile SOM. Shifts in soil-respired $\delta^{13}\text{CO}_2$ over the course of soil incubations were found to reflect changes in labile substrate utilisation. There was a rapid depletion of $\delta^{13}\text{CO}_2$ (from a starting range between -22.5 and -23.9‰, to between -25.8 and -27.5‰) immediately after soil sampling. These initial changes in $\delta^{13}\text{CO}_2$ indicated an increased availability of labile SOM following the disturbance of coring the soil and starting the incubations. Subsequently $\delta^{13}\text{CO}_2$ reverted back to the initial, relatively enriched starting values, but this took several months and was due to labile SOM pools becoming exhausted.

A subsequent study was undertaken to test if soil-respired $\delta^{13}\text{CO}_2$ values are a direct function of the amount of labile SOM and soil physical conditions. A range of pasture soils were incubated in the short-term (300 minutes), and changes in soil-respired $\delta^{13}\text{CO}_2$ were measured along with physical and chemical soil properties. Equilibrium soil-respired $\delta^{13}\text{CO}_2$, observed after the initial rapid depletion and stabilisation, was a function of the amount of labile SOM (measured as hot water extractable C, HWE C), total soil C and soil protection capacity (measured as specific soil surface area, SSA). An independent experimental approach to assess the effect of SSA, where labile SOM was immobilised onto allophane – a clay mineral with large, active surface area – indicated limited availability of labile SOM through more enriched $\delta^{13}\text{CO}_2$ (in a range between -20.5 and -20.6 ‰) and a significant (up to three times) reduction in HWE C.

In the third study, isotopic measurements were coupled with CO₂ evolution rates to directly test whether equilibrium soil-respired $\delta^{13}\text{CO}_2$ can reflect labile SOM vulnerability to loss. Soils were sampled from an experimental tillage trial with different management treatments (chemical fallow, arable cropping and permanent pasture) with a range of C inputs and soil disturbance regimes. Soils were incubated in the short- (300 minutes) and long-term (600 days) and changes in $\delta^{13}\text{CO}_2$ and respiration rates measured. Physical and chemical fractionation methods were used to quantify the amount of labile SOM. Pasture soils were characterised by higher labile SOM estimates (HWEF; sand-sized C; labile C respired during long-term incubations) than the other soils. Long-term absence of plant inputs in fallow soils resulted in a significant depletion of labile SOM (close to 50% based on sand-sized C and HWEF estimates) compared with pasture soils. The values of $\delta^{13}\text{CO}_2$ became more depleted in ^{13}C from fallow to pasture soils (from -26.3 ‰ to -28.1 ‰) and, when standardised (against the isotopic composition of the solid soil material), $\Delta^{13}\text{CO}_2$ values also showed a decrease from fallow to pasture soils (from -0.3 ‰ to -1.1 ‰). Moreover, these patterns in isotopic measures were in strong agreement with the amount of labile SOM and its availability across the soils, and were best explained by the isotopic values of the labile HWEF fraction.

Collectively, these results confirm that labile SOM availability and utilisation change immediately after soil disturbance. Moreover, isotopic analysis of soil-respired CO₂ is a powerful technique, which enables us to probe mechanisms and examine the consequences of soil disturbance on labile SOM by reflecting its availability and the degree of SOM protection.

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Chapter 2: published in *Soil Biology and Biochemistry* (2014, vol. 68, pp. 125-132)

Chapter 3: accepted for publication in the *European Journal of Soil Science*

Chapter 4: under review in the *Soil Science Society of America Journal*

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Certification by Co-authors:

If there is more than one co-author then a single co-author can sign on behalf of all

The undersigned certifies that:

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CHAPTER 1: INTRODUCTION

1.1. Concepts of soil

What is **SOIL**? Is it earth? Is it ground? Is it land? Is it the mud and dirt from a recent aftershock or a flooding event? Or is it, perhaps, the living mantle of the planet Earth?

The term ‘soil’ is indeed perceived differently by various users. Geologists see soil only as a mantle on the Earth’s surface; engineers – as a finely divided rock material; hydrologists – as a storage reservoir affecting the water balance in the catchment; while ecologists are likely to be interested only in the soil properties, influencing growth and distribution of plant species. Farmers are naturally concerned about how soil can influence crops, their growth and yield, as well as the health of the livestock; however, quite often, farmers’ interests do not extend beyond the top 20 – 25 cm of soil – the layer disturbed by a plough and the rooting zone of crop plants. All these views have led to the development of a number of historic and contemporary concepts of soil.

In 1883, Vasily Vasilijevich Dokuchaev, the founder of Russian soil science and pedology, described soils in this way – “soils are the surface mineral and organic formations, always more or less coloured by humus, which constantly manifest themselves as a result of the combined activity of living and dead organisms, parent material, climate, relief and time” (Dokuchaev, 2008). Nowadays, the term ‘soil’ is defined as a complex, three-dimensional, heterogeneous continuum, variable in space and time, which is situated at the interface of four spheres (atmosphere, lithosphere, hydrosphere and biosphere), consisting of four major components (mineral matter, organic matter, water and air) (White, 1997). Soils develop slowly from various parent materials and are modified by time, climate, macro- and micro-organisms, vegetation, and topography. Undoubtedly, soils are very complex structures that interact continuously in response to natural and imposed biological, chemical, and physical forces.

1.2. Why is soil so important?

Soil functions are diverse. Under natural conditions soil serves as a medium for plant growth and a habitat for soil organisms; soil stores and re-cycles nutrients; regulates the quality and distribution of water supply and maintains biodiversity. Furthermore, soil filters, buffers, degrades, immobilises and detoxifies organic and inorganic materials, including industrial pollutants and atmospheric depositions (Blum, 2005). Soil has also made construction and manufacturing possible, by acting as a landscaping and engineering medium. Green roofs or living roofs are a great example of combining environmental and structural benefits. Moreover, soil is a great archaeological diary, as it keeps a detailed record of past environments and enables historic reconstruction. As a result, soils provide ecosystem services that are essential to the well-being of the human population (Filley & Boutton, 2006). Thus, soil can truly be called a ‘life support system of humankind’.

Soils play a key role in the function of the Earth system on the global scale. By being part of the global biogeochemical element cycles, such as the global carbon (C) cycle, soils can modify and ameliorate the risks and effects of climate change. Soils are the largest pool of carbon (C) in the terrestrial C cycle, and according to the recent estimates, in soil organic matter (SOM) they store 1500 Pg C ($1\text{Pg} = 10^{15} \text{ g}$) (Amundson, 2001). This amount is twice as much C as is stored in the atmosphere and nearly triple the amount of C in terrestrial biomass (Amundson, 2001). More importantly, soil C is a dynamic component of the global C cycle, which is closely linked to the atmospheric carbon dioxide (CO_2) pool via inputs from dead organic matter production and losses from decomposition. These CO_2 fluxes are currently 10-fold greater than fossil fuel combustion, so that even small changes in the magnitude of the soil C pool or the input/output rates associated with it could have profound consequences for the global C cycle, the C dioxide concentration in the atmosphere, and the climate system (Lal, 2004; 2008). We are now living at a time of climate change and global

warming, with rising global temperatures and sea levels due to increasing anthropogenic greenhouse gas emissions, which reached 49 (± 4.5) Gt CO₂ eq/ yr in 2010 (IPCC, 2014). Thus, we need to better understand how soils respond to changes in the Earth system. In particular, the main research challenges, prioritised at the beginning of the 21st century as mentioned by Filley and Boutton (2006), focus on biogeochemical cycles, biological diversity and ecosystem functioning, climate variability, and land-use dynamics. These challenges all have SOM dynamics as either an explicit or implicit central issue.

1.3. Soil organic matter... Why do we care?

‘Soil organic matter’ (SOM) is defined as a mixture of plant and animal parts and material that has been altered to the degree that it no longer contains its original structural organization (Oades, 1989; Amundson, 2001). SOM plays a major role in maintaining soil quality and soil fertility by influencing a wide range of soil properties. SOM is a reservoir of nutrients which regulates the supply of inorganic cations (thus buffering pH fluctuations) as well as nitrogen, sulphur and phosphorus. SOM physical properties determine water holding capacity (due to an ability to absorb, hold and release plant-available water), formation and stabilisation of soil structure (through soil aggregates), thus reducing the risk of soil compaction and soil loss with erosion. SOM can deactivate organic chemicals such as herbicides due to sorption capacity. In addition, SOM promotes soil microbial activity by providing a food source for microorganisms (McLaren & Cameron, 1996; Amundson, 2001).

The amount of SOM in soils can vary greatly (with mineral soils worldwide usually storing from 1 to 20% SOM by weight, and organic soils reaching even higher levels (McLaren & Cameron, 1996). In addition, more than a half (on average 58%, with variations across depths and soil types) of SOM is soil carbon (C) (Howard, 1965). The amount of SOM at any given time reflects the long-term balance between C inputs (e.g. fresh plant C inputs)

and losses (e.g. leaching, erosion). These processes are in turn governed by the so-called factors of soil formation which were identified at the early stages of contemporary soil science by Dokuchaev in 1883 (Dokuchaev, 2008) and Jenny in 1941 (Jenny, 1994) and include climate, topographic position, parent material, soil biota, time, and human activity. The latter is becoming more and more important in the constantly changing world. That is why SOM status is often regarded as a strong indicator of environmental changes, soil fertility and land degradation in particular (Doran & Parkin, 1994). However, converting grasslands, forest or other native ecosystems to croplands with intensive agricultural cultivation and soil disturbance leads to SOM loss (Guo & Gifford, 2002), with soils having already lost between 40 and 90 Pg C ($1 \text{ Pg} = 10^{15} \text{ g C}$) globally through cultivation and disturbance (Smith, 2008). Thus, the modern world, building on the past and planning for the future, is strongly focused on SOM sequestration, storage and stabilisation. However, one of the most intriguing properties of SOM is its dynamic nature, as SOM is most useful in its decay. So, as pointed out by Janzen (2006), we face a SOM dilemma: “Shall we hoard it or use it? Can we both conserve SOM and benefit from its decay? Or must we choose one or the other?” In order to resolve the dilemma we need to focus our research efforts on improving our understanding of SOM dynamics.

1.4. SOM as a complex continuum

While contemporary concepts of sustainable agriculture emphasize the importance of adequate SOM management for future soil fertility (Conway & Barbier, 1990), the development of the SOM concept as we know it today has not been easy. Despite the fact that we cannot date the first occurrence of the term ‘soil organic matter’ in science with any precision, it is possible to identify three periods in the scientific perception of SOM (Manlay

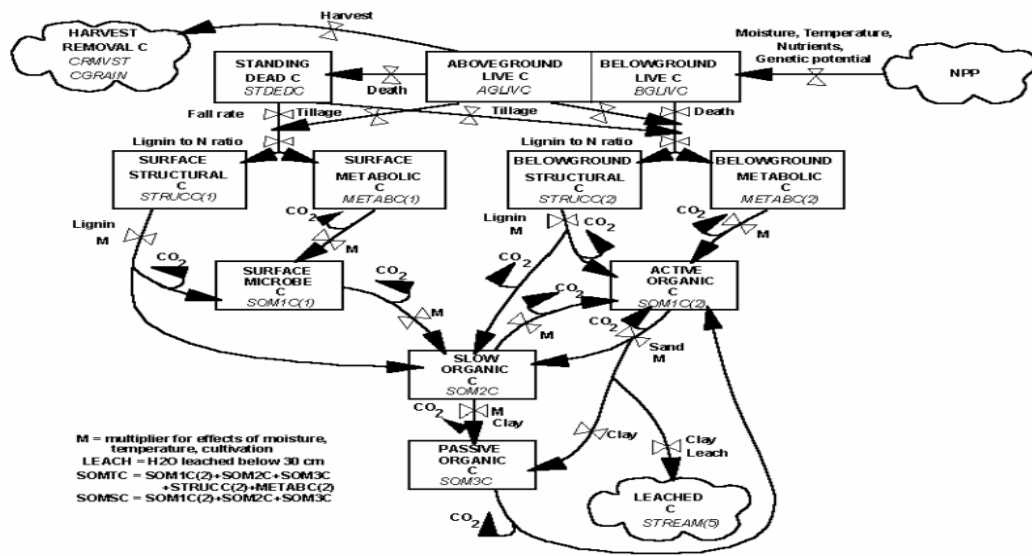
et al., 2007). The ‘humic period’ lasted until 1840s with the term ‘humus’, as a SOM precursor, known since the Roman times; the ‘mineralist period’ was dominated by the Liebig’s ‘mineral nutrition theory’ and development of intensive mineral fertilization. The third ‘ecological period’ started in 1940s and was characterized by a widening of the SOM perception, with its recognition as a complex bio-organo-mineral system and an indicator of soil quality (Manlay *et al.*, 2007). The second half of the 20th century was characterised by significant developments in the area of soil science and SOM in particular. Application of new analytical techniques (e.g., spectroscopy, chromatography, analytical pyrolysis, nuclear magnetic resonance) have led to confirmation of the complex SOM structure as a mix of molecules of varying polymericity and aromaticity (e.g. carbonyl, aromatic, O-alkyl and alkyl groups) (Skjemstad *et al.*, 1997). However, clarifying the SOM nature and dynamics at a detailed process level still remains a major scientific challenge. SOM has become recognised as an ecosystem compartment with its links and functions following the development of the concept of ‘biocoenosis’ (or ‘geobiocoenosis’) by the pioneering Russian soil scientist Dokuchaev (2008) and his followers in the late 19th century; and a practically identical term ‘ecosystem’ by Tansley in 1935 (Manlay *et al.*, 2007). Furthermore, due to a large amount of SOM in soils worldwide, now soils are perceived as very important C sinks capable of influencing global biogeochemical cycles (Amundson, 2001).

The modern perception of complex SOM as a mixture of pools or fractions with different turnover rates and decomposability is the result of the combination of chemical and ecological approaches to study SOM. As a result, a number of methods separating and quantifying SOM pools and fractions have been invented. Historically, the advances in inorganic chemistry have made the chemical study of SOM possible; however, it has proved to be much more complex than that of inorganic chemical compounds. Soil humic acids were first extracted in the 18th century; and the contemporary classification of extracted humus

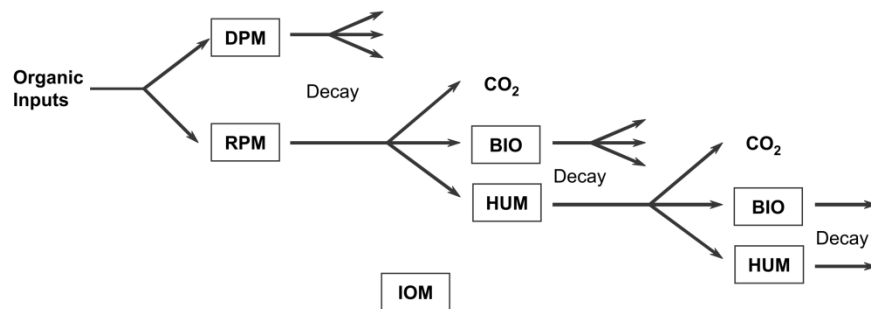
materials (into humic acid, fulvic acid, hyalomelanic acid, and humin) was proposed by Oden in 1919, and was later modified by Waksman in 1936 (Schulthess, 2011). However, this classification is operationally defined and is based on extraction with concentrated solutions of NaOH, which are known to modify SOM (Schulthess, 2011).

In order to simplify challenging SOM dynamics studies, contemporary soil modellers have come up with a number of SOM models. These simulation models, which are derived from the principles of decomposition science, are widely used nowadays to predict SOM pool sizes and their dynamics at the plot, ecosystem or even regional scale (Sharifi *et al.*, 2013). The conventional SOM models usually include one to two labile and/or dynamic pools, two to three physically and chemically protected pools, and one passive or even inert pool, usually defined by fixed decomposition rates. For example, in the CENTURY model (**Figure 1.1A**) the SOM continuum has been divided into three discrete conceptual pools based on the turnover times: (1) **highly labile or active pool**, which accounts for only 5% of SOM and includes such easily decomposable compounds as fresh plant material and root exudates with residence times of ~ 1 year, (b) **slow or intermediate pool**, with residence times of $10^0 - 10^2$ years, which represents 60 – 85 % of SOM and (c) the **passive or recalcitrant pool**, which accounts for 10 – 40 % of SOM, and due to chemical recalcitrance and various lack of microbial access can remain in soils for millennia (Parton *et al.*, 1987; Parton *et al.*, 1992).

A CENTURY model



B ROTH-C model



RPM : Resistant Plant Material
DPM : Decomposable Plant Material
BIO : Microbial Biomass

HUM : Humified OM
IOM : Inert Organic Matter

Figure 1.1. Conventional SOM models: A – CENTURY (after Parton *et al.* (1992), B – ROTH-C (after Coleman and Jenkinson (1999). See the text for definitions.

Another well-known conventional model is the ROTH-C model, which partitions SOM into five pools (**Figure 1.1B**). Inert organic matter (IOM) has a nominal radiocarbon age of 50 000 years. Incoming plant material is separated into decomposable (DPM) and resistant (RPM) plant material with an empirically validated DPM:RPM ratio of 1.44 for arable and grassland soils. The plant material decomposes to form CO₂, biomass (BIO) and humified organic matter (HUM). BIO and HUM are further decomposed to form more CO₂, BIO and HUM. All pools except IOM decompose by first-order decay at rates of 10 y⁻¹ for DPM, 0.3 y⁻¹ for RPM, 0.66 y⁻¹ for BIO and 0.02 y⁻¹ for HUM. (Jenkinson & Rayner, 1977; Coleman & Jenkinson, 1999). Davidson and Janssens (2006) present a visual comparison of the properties of SOM pools from the CENTURY and ROTH-C models (**Figure 1.2**). As mentioned above, the three discrete SOM pools are characterised by different decomposability and turnover rates in soils.

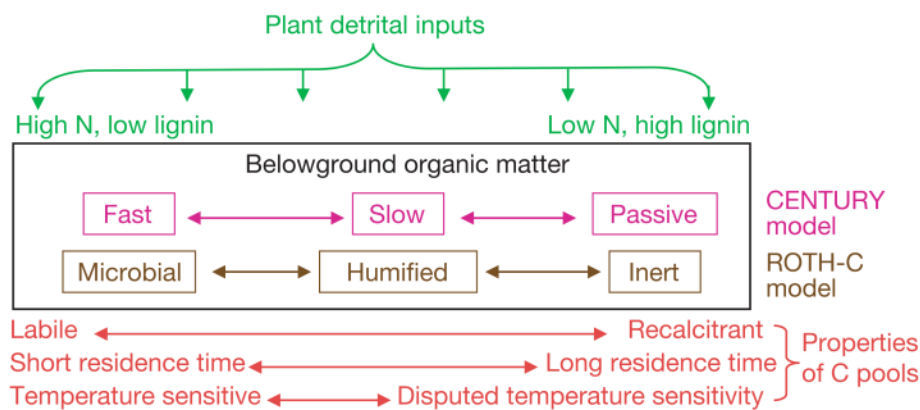


Figure 1.2. Comparison of conceptual SOM pools in the CENTURY and ROTH-C models (after Davidson and Janssens (2006)).

In contrast to modelling, various procedures of the soil analysis, based on chemical, physical or biological fractionation or a combination, are used to physically separate SOM pools before measurement (see review by von Lützow *et al.* (2007)). Water soluble carbon (WSC) and hot water extractable carbon (HWEC) are common measures of labile SOM, based on the assumption that SOM that is easily degradable by microbial enzymes is more soluble in water than other organic fractions in the soil and is easily extractable (McLauchlan & Hobbie, 2004). Other chemical methods include stronger treatments: acid hydrolysis (with HCl or H₂SO₄), oxidation with potassium permanganate (KMnO₄) or hydrogen peroxide (H₂O₂). These methods extract a higher proportion of total soil C content, not just easily available SOM (see review by von Lützow *et al.* (2007)). Non-hydrolysable SOM is often considered to be a meaningful estimate of the passive pool in the CENTURY model, based on pools sizes and ages (Paul *et al.*, 1997). Hydrofluoric acid (HF) is used to destroy the mineral phase and isolate SOM from the organo-mineral associations; it is known to dissolve up to 80% of total SOM (see review by von Lützow *et al.* (2007)).

Physical fractionation methods are based on the concept that SOM fractions associated with different size particles (e.g., sand, silt and clay) or different densities (e.g., light and heavy fractions) have different structures and functions (Christensen, 2001). Sand-associated SOM and the light fraction represent only a small proportion (often <10%) of total SOM (Cambardella & Elliott, 1992; Christensen, 2001). However, these SOM fractions are rapidly mineralised, can be quickly affected by changes in management practices, and are widely regarded as labile SOM fractions (Balesdent, 1996). Clay-associated SOM and the heavy SOM fraction, on the other hand, are considered to represent the stable SOM pool.

Biological fractionation assumes that microbes mineralize labile SOM first, and separates the pools by allowing microbes to mineralize SOM without fresh organic inputs (Townsend *et al.*, 1997; Alvarez & Alvarez, 2000; McLauchlan & Hobbie, 2004). The

technique involves measuring CO₂ produced during the course of laboratory incubations of soil under controlled conditions and quantifying the size of the labile SOM pool based on the initial CO₂ flux (Townsend *et al.*, 1997; Alvarez & Alvarez, 2000; McLauchlan & Hobbie, 2004). The drop and stabilisation of soil respiration at a steady rate with time is attributed to a near complete loss of active, labile SOM; with a steady CO₂ flux being determined by the respiration of a more recalcitrant SOM pool (Townsend *et al.*, 1997). Another biological fractionation method is quantification of the soil microbial biomass using the chloroform-fumigation and extraction technique (Jenkinson, 1976). Soil microbial biomass is often used as another measure of the labile fraction; moreover, it is considered to be the main component of the active SOM pool and has been linked to the “eye of a needle” through which carbon and nutrient transformations are mediated (Jenkinson, 1976).

So, there are a number of different laboratory fractionation procedures to isolate and quantify SOM pools. However, we should not forget that SOM is a complex continuum, and it is quite challenging to establish clear equivalence between the measured fractions and the outputs from the model simulations. A number of researchers have tried to ‘model the measurable’ or/and ‘measure the modelable’ (Elliott *et al.*, 1996). For example, Zimmermann *et al.* (2007) have related measured SOM fractions to pools in the ROTH-C model. Other researchers have come up with conceptual models of SOM dynamics with measurable pools. **Figure 1.3A** illustrates the model suggested by Six *et al.* (2002). Mechanistic soil processes are incorporated in the model but the conceptual pools (active, slow and passive) developed in the conventional models (Jenkinson & Rayner, 1977; Parton *et al.*, 1987), are still reflected. The authors hypothesized that the micro-aggregate-protected C plus silt- and clay-protected C represents part of the slow pool whereas the unprotected pool represents the active fraction and part of the slow pool. The non-hydrolysable fraction of the silt- and clay-associated C represents the biochemically protected pool and is hypothesized to be

comparable to the passive pool. The unprotected C pool is POM or LF not occluded within micro-aggregates whereas the micro-aggregate-protected C pools is fine POM occluded within micro-aggregates (**Figure 1.3A**, Six *et al.* (2002). Another model with measurable pools, called AggModel (**Figure 1.3B**) has been recently developed by Segoli *et al.* (2013). The model recognises four unique physical pools: unaggregated soil external to macro-aggregates ($> 250 \mu\text{m}$), micro-aggregates ($< 250 \mu\text{m}$) external to macro-aggregates, micro-aggregates within macro-aggregates and non-micro-aggregated soil within macro-aggregates (**Figure 1.3B**). Within each of the pools the authors distinguished between two SOM fractions: particulate organic matter (POM) with organic matter fragments $> 53 \mu\text{m}$ in various stages of decomposition (Cambardella & Elliott, 1992); and mineral-associated organic matter (MAOM), which is more transformed and degraded organic matter, not easily separated from small mineral particles $< 53 \mu\text{m}$ (Segoli *et al.*, 2013). Despite these recent advances in approach, SOM complexity is still creating challenges for researchers.

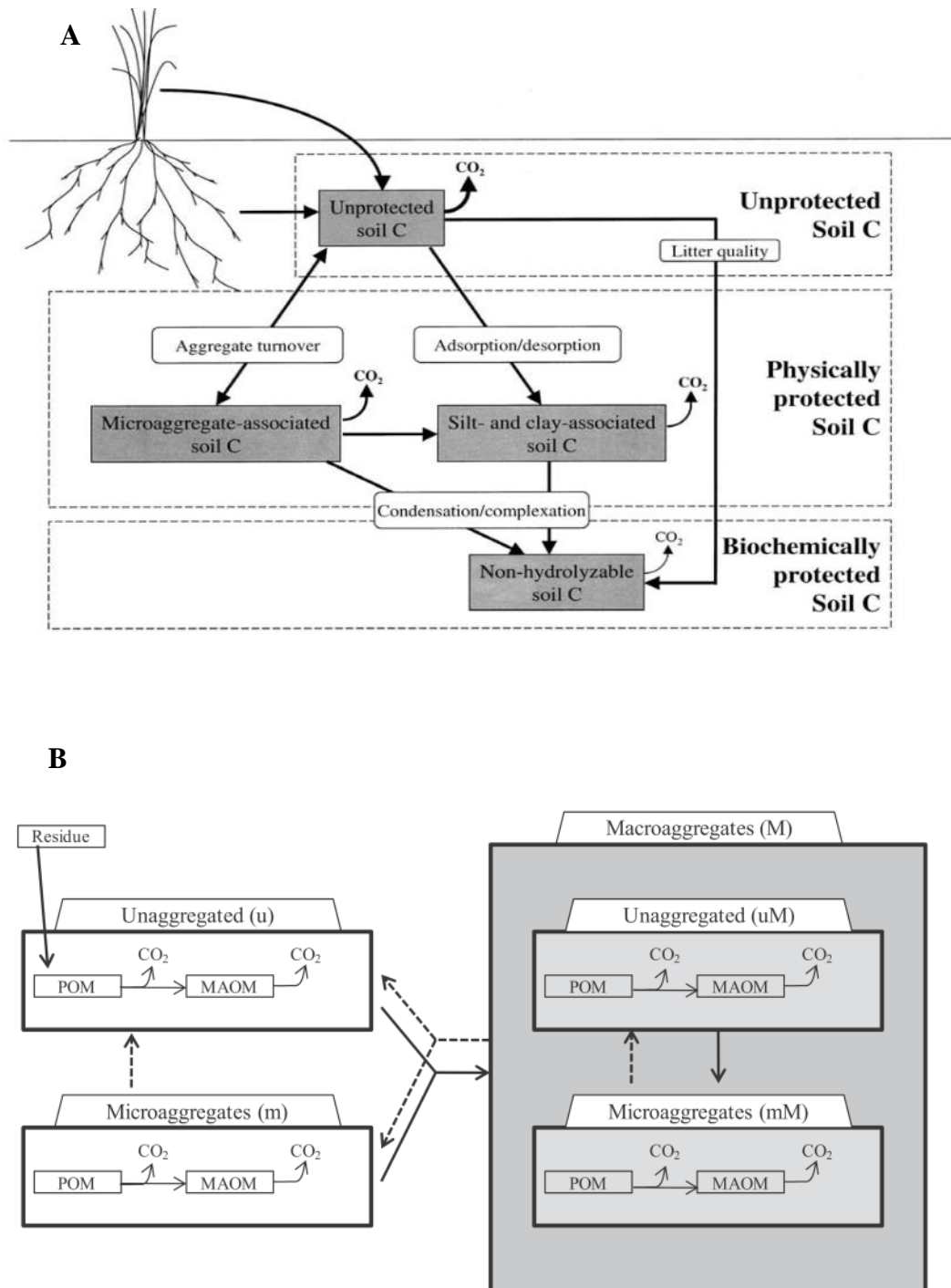


Figure 1.3. Conceptual models of SOM dynamics with measurable pools. A – model after Six *et al.* (2002). B – AggModel, after Segoli *et al.* (2013). See the text for definitions.

1.5. An alternative approach... The use of carbon isotopes

An alternative approach for quantifying SOM dynamics can be the use of C isotopes. The term '*isotope*' is formed from Greek roots ('*the same place*') and means a variant of a particular chemical element. All isotopes of a given element occupy the same position in the Periodic Table, have the same number of protons in each atom, but differ in neutron number. The number of nucleons (both protons and neutrons) in the nucleus is the atom's mass number, and each isotope of a given element has a different mass number. So, for C as a chemical element ^{12}C , ^{13}C and ^{14}C will be the three most common isotopes with mass numbers 12, 13 and 14 respectively. The atomic number of C is 6, which means that every C atom has 6 protons, so that the neutron numbers of these isotopes are 6, 7 and 8 respectively. ^{12}C and ^{13}C are stable C isotopes with ^{12}C being the dominant one (98.89%), whereas the natural abundance of ^{13}C is only 1.11%. ^{14}C is a radioactive isotope as it undergoes radioactive decay at a defined rate with a certain half-life time (5700 yr). The isotopic ratio – the isotopic signature (or isotopic fingerprint) – is usually reported in parts per thousand (per mil, ‰) and expressed as a delta notation (δ or Δ) relative to an isotope-specific standard, using the equation outlined by McKinney *et al.* (1950):

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (1.1)$$

where R stands for the ratio between the isotopes of interest.

Recently, C isotopes have become the basis of a popular approach to trace C fluxes between the atmosphere, vegetation and soil as reviewed by Dawson *et al.* (2002) and Paterson *et al.* (2009).

1.5.1. Radio-carbon approach

Testing of nuclear weapons in the 1950s and early 1960s has allowed researchers to use ‘bomb ^{14}C ’ as a method for radiocarbon dating to quantify the turnover of SOM pools on a timescale of years to decades (Gaudinski *et al.*, 2000; Trumbore, 2000; 2006). On this timescale radioactive decay is negligible, and ^{14}C acts as a conservative tracer. The atmospheric ^{14}C levels nearly doubled between 1955 and 1964, reaching +1000 ‰ in 1964. Since the end of weapons testing, the bomb ^{14}C signal has been declining each year (by 5 – 10 ‰ per year, as mentioned by Trumbore (2006), due to burning of ^{14}C -free fossil fuels and atmospheric CO_2 exchanges with terrestrial and marine C reservoirs. Different residence times of C within the ecosystem result in different radiocarbon signatures of SOM pools in the soil: the degree to which the bomb ^{14}C is found in the SOM gives a measure of the degree to which soils have incorporated C fixed from the atmosphere over the past 50 years. Recent photosynthetic products have $\Delta^{14}\text{C}$ values equal to contemporary atmospheric CO_2 ; while CO_2 respired by SOM with C fixed years to decades ago will have elevated ^{14}C signatures, e.g. > 100 ‰ (Trumbore, 2000). Low values (< 0 ‰) show that SOM has been in the soil for long enough (> 300 years) for significant radioactive decay of ^{14}C to have occurred. Intermediate values represent a mixture of SOM pools with fast and slow turnover rates. So, ^{14}C provides a tool to quantify the turnover of SOM pools on a timescale of years to decades, and separate ‘recent’ from ‘older’ sources of respiration (Trumbore, 2000; 2006). Another advantage of the method is in its ability to be used both in disturbed and undisturbed ecosystems. However, the radiocarbon in the solid phase can provide misleading estimates of the short-term SOM response as it cannot estimate decomposition of the labile SOM pool with very fast (< 1 year) turnover times (Trumbore, 2000; 2006). So, the radiocarbon approach may work well in peaty soils, where decomposition is slow and SOM has a large stable component, which can be preserved in soils for a long time. However, this method may

not be as reliable in ecosystems with annual plants where the proportion of active, labile SOM is reasonably high (Trumbore, 2000; 2006).

1.5.2. Stable C isotope approach

An alternative approach to radiocarbon is based on the use of stable C isotopes. The isotopic signature ($\delta^{13}\text{C}$) is expressed as a delta notation using the equation mentioned above (Equation (1.1)). The reference for the ^{13}C isotope is the Vienna Pee Dee Belemnite standard, which is based on a Cretaceous marine fossil (*Belemnitella Americana*) from the Pee Dee Formation in South Carolina. This material has an anomalously high $^{13}\text{C}/^{12}\text{C}$ ratio (0.0112372), so it has been assigned a value of zero. Use of this standard gives most natural material a negative $\delta^{13}\text{C}$. The delta notation ($\delta^{13}\text{C}$) is also reported in per mil (‰), due to low natural abundance of ^{13}C .

One use of the stable C isotopes approach involves continuous or pulse-labelling with a label of a different signature. A number of authors have used pulse-labelling to study SOM dynamics: a highly enriched or naturally depleted in ^{13}C isotopic label has been often used in FACE (Free Air CO_2 Enrichment) studies, which facilitates estimation of SOM pool sizes, C sequestration and residence times of SOM and its fractions (Pendall *et al.*, 2004; van Kessel *et al.*, 2006; Pendall & King, 2007; Dorodnikov *et al.*, 2011).

Another variation of the method is based on the differences in the isotopic composition of atmospheric CO_2 and $\delta^{13}\text{C}$ values of plants with different types of photosynthesis. The current $\delta^{13}\text{C}$ signature of the CO_2 in the atmosphere is approximately -8 ‰, whereas 30 – 100 years ago it was -6.5 ‰ (Ehleringer *et al.*, 2000). This depletion in the ^{13}C values of the atmospheric CO_2 is mainly due to combustion of ^{13}C -depleted fossil fuels since the industrial revolution, as reviewed by Ehleringer *et al.* (2000). Differential discrimination of the heavier ^{13}C isotope during CO_2 assimilation by plants with different

types of photosynthesis leads to natural differences in isotopic composition (Boutton *et al.*, 1998; Bowling *et al.*, 2008). In C₃ plants the primary carboxylating enzyme (*rubisco*) heavily discriminates against ¹³C, leading to δ¹³C values of plant tissues of about -27 ‰ (-35 ‰ ≤ δ¹³C ≤ -20 ‰) (Boutton *et al.*, 1998). In C₄ plants the primary carboxylating enzyme (*phosphoenol pyruvate carboxylase*) discriminates to a lesser extent, therefore, δ¹³C values of C₄ plants are less depleted in ¹³C (-15 ‰ ≤ δ¹³C ≤ -7 ‰) (Boutton *et al.*, 1998). These large differences in the isotopic signatures and differential discrimination pathways have allowed study of SOM dynamics and assessment of the proportion of recently fixed labile SOM *versus* relatively recalcitrant, older SOM pool in ecosystems following a transition between C₄ and C₃ vegetation. An example of this is growing maize after cutting down a C₃ forest or converting a ryegrass – white clover pasture (Balesdent *et al.*, 1987; Balesdent *et al.*, 1988; Balesdent, 1996; Cadisch *et al.*, 1996; Balesdent *et al.*, 1998; Hanson *et al.*, 2000; Zingore *et al.*, 2005; Virto *et al.*, 2010; Blagodatskaya *et al.*, 2011).

Recently, it has been demonstrated that differences in isotopic composition occurred in ecosystems naturally, without isotopic labelling or C₃ – C₄ plant transition (Ehleringer *et al.*, 2000; Bowling *et al.*, 2008). As demonstrated in these reviews, the isotopic signature of the SOM is commonly enriched compared with the associated litter and leaves of the standing vegetation (**Figure 1.4**). Therefore the δ¹³CO₂ signature of CO₂ respired by SOM (δ¹³CO₂) is typically 2 – 4‰ enriched, compared with the respiration of roots and their associated microbes (Bowling *et al.*, 2008). Moreover, SOM deeper in a soil profile is more enriched in ¹³C, compared with the surface layer (**Figure 1.4**). Preferential microbial decomposition and

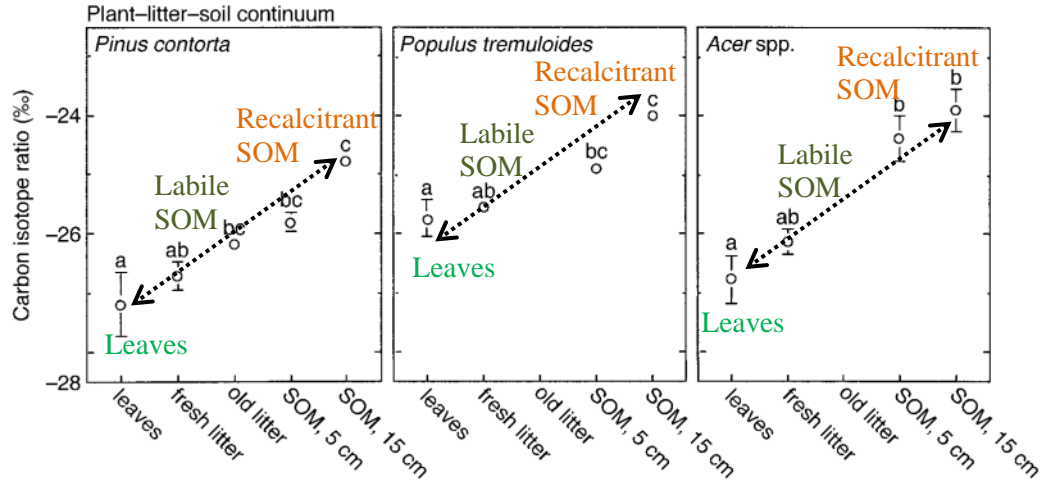


Figure 1.4. Isotopic signatures of leaves, fresh and old litter, and SOM at 5 and 15 cm depths (after Ehleringer *et al.* (2000)).

microbial fractionation along with SOM mixing and the influence of atmospheric change are likely to be responsible for this enrichment trend (Ehleringer *et al.*, 2000). Labile SOM consists of relatively fresh plant residues which have been less processed by microbes, which discriminate against the heavy ¹³C isotope (Ehleringer *et al.*, 2000). Thus labile SOM is relatively depleted in ¹³C and can be characterised by the isotopic signatures closer to the values of plant tissues (in case of C₃ vegetation approximately -27 ‰). More recalcitrant SOM has undergone a number of cycles of microbial fractionation (Ehleringer *et al.*, 2000), and thus it has become relatively enriched in ¹³C as soil microbes have preferentially used up the lighter ¹²C isotope (Figure 1.4). These differences in isotopic composition allowed Gunina and Kuzyakov (2014) to study C flows between various SOM fractions. They observed progressive ¹³C enrichment between the SOM fractions, with the most labile fraction being close to initial plant material and depleted in ¹³C and the mineral fraction being most processed and enriched in ¹³C. Millard *et al.* (2010) measured ¹³C signatures of soil-respired CO₂ and found rapid changes in soil-respired δ¹³CO₂ within a few hours since disturbing the soil by soil sampling. The authors suggested that the shift in the isotopic

signatures might be the result of SOM dynamics and rapid changes of its labile component in particular. This recent observation created the foundation for this thesis.

1.6. Thesis hypotheses:

My thesis aimed to improve understanding of the SOM dynamics, and the specific response of its labile component to soil disturbance. I was particularly motivated to use the natural abundance ^{13}C isotopic approach. This thesis has addressed the question *can the isotopic values of soil-respired CO_2 be used as a measure of the amount of labile SOM, its vulnerability and degree of protection from loss following soil disturbance?*

The following **objectives** formed the basis for this research:

1. To determine the effect of soil disturbance on labile SOM and labile C substrate utilisation by recording the shifts in soil-respired $\delta^{13}\text{CO}_2$ immediately after disturbing the soil and over the course of long-term soil incubations.
2. To determine if the short-term changes in soil-respired $\delta^{13}\text{CO}_2$ are a direct function of the amount of labile SOM and soil physical conditions. Models were fitted to identify which combination of soil properties and traditional measures of labile SOM best explained the observed equilibrium soil-respired $\delta^{13}\text{CO}_2$. Additionally, the role of soil surface area in determining labile SOM availability and protection from loss was investigated.
3. To determine if the isotopic composition of soil-respired CO_2 is a measure of labile SOM vulnerability to loss, following soil disturbance. In addition, effects of long-term regular C inputs and mechanical soil disturbance regimes on labile SOM pools, their vulnerability and protection from loss were assessed.

1.7. Thesis outline:

This thesis addresses the question can the isotopic values of soil-respired CO₂ be used as a measure of the amount of labile SOM, its vulnerability and degree of protection from loss following soil disturbance?

In the introduction (**CHAPTER 1**) I describe the importance of soils and soil organic matter (SOM), present an overview of SOM research and various approaches for measuring the complex SOM body. This chapter also sets up the scene, outlines the aims and the overview of the research presented in this thesis.

In **CHAPTER 2** I present the results of the short- and long-term soil incubations carried out to test the hypothesis that *the shifts in soil-respired $\delta^{13}\text{CO}_2$ over the course of soil incubations are due to changes in labile C substrate utilisation* (Hypothesis 1). The work presented in this chapter has been published in *Soil Biology and Biochemistry* (2014, vol. 68, pp. 125-132). A copy of the paper can be found in **APPENDIX # 1**.

Building on these findings, in **CHAPTER 3** I test the hypothesis that *during soil incubations, short-term changes in soil-respired $\delta^{13}\text{CO}_2$ are a direct function of the amount of labile SOM and soil physical conditions* (Hypothesis 2). I fit models to determine which combination of soil properties (and various traditional measures of labile SOM in particular) best explained the observed equilibrium soil-respired $\delta^{13}\text{CO}_2$. Additionally, I assess in more detail the role of soil surface area in determining labile SOM availability and protection from loss. The work presented in this chapter has been accepted for publication in the *European Journal of Soil Science*.

In **CHAPTER 4** I test the hypothesis that *the isotopic signatures of soil-respired CO₂ are a measure of labile SOM vulnerability to loss, following soil disturbance* (Hypothesis 3). I test if the proportion of labile C respired over the course of the incubations (as a traditional measure of labile SOM pool based on soil respiration) provides the best predictive value of the isotopic signatures of soil-respired CO₂. In addition, I assess the effects of long-term regular C inputs and mechanical soil disturbance regimes on labile SOM pools, their vulnerability and protection from loss and make links with the SOM stabilisation theory. The work presented in this chapter is under review in the *Soil Science Society of America Journal*.

Finally, **CHAPTER 5** is a synthesis of the three previous experimental chapters, where I summarise our improved understanding of labile SOM dynamics. I compare various traditional methods of quantifying labile SOM pool with the new isotopic approach and discuss the possibilities for a potentially quick method of assessing SOM vulnerability to loss and degree of SOM protection. I suggest links with the SOM stabilisation theory and propose similarities with the soil C saturation curve. Additionally, I point out the directions for further research on a variety of soil types and under a number of management treatments.

**CHAPTER 2: LOSS OF LABILE CARBON FOLLOWING
SOIL DISTURBANCE DETERMINED BY MEASUREMENT
OF SOIL-RESPIRED $\delta^{13}\text{CO}_2$**

Published in *Soil Biology and Biochemistry* (2014, vol. 68, pp. 125-132)

2.1. Introduction

Soils are the largest pool of carbon (C) in terrestrial ecosystems, globally containing more than two-thirds of ecosystem total C (Amundson, 2001). Land-use change and any accompanying soil disturbance can be a major cause of loss of soil C, for example following deforestation (Cadisch *et al.*, 1996; Guo & Gifford, 2002; Zingore *et al.*, 2005), cultivation (Elliott, 1986) or cropping (Guo & Gifford, 2002). Labile, soil organic matter (SOM) is considered to have fast decomposition rates and short turnover times. It accounts for only about 5% of SOM, but is highly active as it consists of easily decomposable compounds (Townsend *et al.*, 1997; Krull *et al.*, 2003). It can be vulnerable to microbial degradation due to lack of stabilisation onto clay minerals or lack of physical protection by soil aggregates (Krull *et al.*, 2003). Such protection occurs due to a range of factors, including reduced oxygen diffusion into soil aggregates and physical separation from soil microbes (Six *et al.*, 2002). Systematic loss of soil C following mechanical soil disturbance is attributed to loss of physically protected SOM (Six *et al.*, 2002). Microbial access to soil C is hypothesised to play a larger role in regulating SOM turnover than molecular recalcitrance (Dungait *et al.*, 2012).

A range of methods have been used to measure labile soil C pools, relying on chemical, physical or biological separation from recalcitrant SOM (McLauchlan & Hobbie, 2004). While results from different methods are positively correlated, they can produce large differences in estimates of labile C pools (McLauchlan & Hobbie, 2004). Dissolved organic C (DOC) and hot water extractable C (HWE) are common measures of chemically extractable C in soils which can be used to estimate the size of soluble labile soil C pools. Positive correlations have been observed between DOC and total soil C, HWE and total soil C, as well as between DOC and HWE (Ghani *et al.*, 2003). HWE is a small pool of labile

C, generally representing 3-6% of total soil C, and has been shown to be a sensitive indicator of changes in organic matter due to soil management (Ghani *et al.*, 2003).

A refinement to the fractionation of SOM for separation of labile from recalcitrant C pools has been the use of ^{13}C signatures to measure loss of recently assimilated C. This approach has relied upon inputs of C into soil with contrasting isotopic signatures, either through a transition between C_4 and C_3 vegetation (Cadisch *et al.*, 1996; Zingore *et al.*, 2005; Virto *et al.*, 2010; Blagodatskaya *et al.*, 2011) or supplying elevated carbon dioxide (CO_2) with a ^{13}C signature different from air thus providing a label (Pendall & King, 2007). In both cases, the distinct difference in ^{13}C signature between the inputs of newly fixed C and the SOM provides a way to differentiate relatively labile *versus* relatively recalcitrant C pools. Measurement of the isotopic signatures of whole soils (Zingore *et al.*, 2005), isolated SOM fractions (Cadisch *et al.*, 1996; Virto *et al.*, 2010; Blagodatskaya *et al.*, 2011), or respired CO_2 (Townsend *et al.*, 1997; Crow *et al.*, 2006; Pendall & King, 2007) has then been used to calculate the residence time of C.

In a study of the $\delta^{13}\text{C}$ signature of soil respiration, Millard *et al.* (2010) found a rapid depletion of $\delta^{13}\text{CO}_2$ within a few hours of soil incubation. These changes in $\delta^{13}\text{CO}_2$ were ascribed to disturbance (as a result of extracting soil cores and removal of roots), causing small but relatively labile soil C pools, previously protected from microbial activity, to become available as respiratory substrates (Millard *et al.*, 2010). In a longer-term study Crow *et al.* (2006) found that, during incubation of different SOM fractions from a forest soil, there was an initial isotope depletion of the respired CO_2 , which subsequently (over a 25 d period) became more enriched, more closely reflecting the value of the solid sample. Crow *et al.* (2006) suggested that one reason for these changes in the isotopic signature of respiration through time was loss of the more labile C pools in the soil that were relatively depleted in

^{13}C . The present study extends this approach, to test the hypothesis that shifts in $\delta^{13}\text{CO}_2$ over the course of soil incubations were due to changes in labile C substrate utilisation.

A range of soils were incubated in both short- and long-term experiments and $\delta^{13}\text{CO}_2$ measured in order to determine if: (1) rapid changes in $\delta^{13}\text{CO}_2$ following soil disturbance were due to release of labile C, (2) long-term incubations resulted in $\delta^{13}\text{CO}_2$ reverting back to original values as labile C pools were exhausted, (3) large scale soil perturbation had an effect on the time needed for $\delta^{13}\text{CO}_2$ to revert back in the long-term incubations. Additional soil was incubated with sand (as a control treatment) and allophane, which due to its large active surface area adsorbs SOM by forming Al-organic complexes (Baldock & Skjemstad, 2000; Yuan *et al.*, 2000), in order to determine if $\delta^{13}\text{CO}_2$ can indicate the loss of labile soil C.

2.2. Materials and methods

2.2.1. Soils

Site 1: Kānuka stand

Soil was sampled from a stand of kānuka (*Kunzea ericoides* (A. Rich) J. Thompson) trees 6m high in Lincoln, New Zealand (43° 38' S, 172° 29' E, 11 m above sea level) which was planted in 1984 (Harris, 1996). Previously, the site was temperate grassland dominated by C_3 grass species. The soil was a Wakanui silt loam (Hewitt, 2010) (USDA classification, Aquic Haplustept), with a litter layer of variable depth (25 – 70 mm) and an H1 horizon to 180 mm depth. The soil is described in detail by Watt and Burgham (1992).

Site 2: Montane grassland

Soil was sampled from a tussock grassland with a mix of native tussocks (*Festuca novae-zelandiae* (Hack.) Cockayne) and pasture species (*Agrostis capillaris* L.) (Burrows, 1977) at the University of Canterbury Cass Field Station in the Central South Island, New Zealand (43° 02' S, 171° 46' E, 590 m above sea level). Soils at this site were classified as

Acidic Allophanic Brown (Hewitt 2010) (USDA classification, Typic Dystruchrept). Samples were taken from two different treatment areas at this site: 1) the perturbed plots of a soil warming experiment and 2) an adjacent undisturbed area of tussock grassland. Establishment of the soil warming experiment involved significant large scale soil perturbation: the top 300 mm of soil was excavated with heavy machinery in late 2008 – January 2009 (one year prior to sampling), left at the site for several months and redistributed after installation of the heating cables. Samples were only taken from the plots, which received the soil perturbation, but were not subjected to warming.

Site 3: Arable cropping

Soil was sampled in the Millennium Tillage Trial (MTT), a field site established by Plant and Food Research in November 2000 on a Wakanui silt loam (Hewitt, 2010) (USDA classification, Aquic Haplustept) near Lincoln, New Zealand (43° 40' S, 172° 28' E, 5 m above sea level). Soil was sampled from replicated no tillage plots sown with barley (*Hordeum vulgare* L.) and also plots which had been maintained under a permanent pasture of ryegrass (*Lolium perenne* L.) and clover (*Trifolium repens* L.).

Site 4: Peatland

Soil was sampled from Middlemuir Moss, a former raised mire site in Scotland (57° 36' N, 2° 9' W, 110 m above sea level). The site has a long history of manual and mechanised peat cutting; up to 4 m of peat has been removed during mechanised harvesting operations between 1961 and 1995. The remaining peat is highly acidic (pH 3.0), humified and between 2 to 4 m deep. No restoration has been carried out and the site is unmanaged. Surface peat exposed after harvesting was used for the experiment and because of its original depth was probably several thousand years old. The vegetation was characterised by patchy spontaneous regeneration with typical mire species such as *Calluna vulgaris* L., *Eriophorum vaginatum* L. and *E. angustifolium* Honck., with scattered patches of *Sphagnum auriculatum* Schimp. and

extensive areas of bare peat (Trinder, 2007; Trinder *et al.*, 2008b). Soil at the site was classified as drained oligotrophic amorphous peat (Acid Humic Organic soil (Hewitt, 2010), USDA classification, Cryohemist). Soil was only sampled from areas containing no vegetation.

Site descriptions are summarised in **Table 2.1**.

Table 2.1. Site descriptions

Site	Kānuka stand	Montane grassland	Arable	Peatland
Latitude, longitude	43° 38' S 172° 29' E	43°02' S 171°46' E	43°40' S 172°28' E	57° 36' N 2° 9' W
Altitude	11 m a.s.l. ^a	590 m a.s.l.	5 m a.s.l.	110 m a.s.l.
Annual rainfall	684 mm	1300 mm	684 mm	748 mm
MAT ^b	11.4° C	9.0° C	11.4° C	8.8° C
Soil type (NZ classification)	Wakanui silt loam	Acidic Allophanic Brown	Wakanui silt loam	Acid Humic Organic soil
Soil type (USDA)	Aquic Haplustept	Typic Dystruchrept	Aquic Haplustept	Cryohemist

^aa.s.l. – above sea level

^bMAT – mean annual temperature

2.2.2. Soil sampling and incubations

The protocol used for soil sampling was similar to the one described by Millard *et al.* (2010). At each site and for each treatment replicate 50 mm diameter soil cores taken to a depth of 250 mm were broken open and visible roots and any stones quickly (within minutes) removed by hand and discarded. All the remaining soil from each core was mixed and placed in a Tedlar[®] bag (Keika Ventures, Chapel Hill, NC, USA). The bag was sealed, and then quickly flushed with CO₂-free air repeatedly (typically 5 - 6 times) until less than 20 ppm CO₂ remained in the bag. Samples were incubated *in situ* at an ambient temperature and aliquots of gas were regularly removed to check the CO₂ concentration with a portable infra-red gas analyser IRGA EGM-4 (PP Systems, Hitchin UK). Once a minimum of 350 ppm CO₂ had accumulated (typically after 7-10 min), the headspace gas was transferred to a second Tedlar bag that had previously been flushed twice with CO₂-free air, and then analysed for $\delta^{13}\text{CO}_2$. The bag with the soil was then flushed with CO₂-free air again and re-incubated until sufficient CO₂ had accumulated for a second sample. This was repeated until the isotopic signature of the respired CO₂ had stabilised.

The soil samples were taken back to the laboratory and weighed. The following day, samples were flushed, incubated and sampled as described above. This was repeated once or twice a week for extended periods, until $\delta^{13}\text{CO}_2$ had once again stabilised. The samples were incubated in the bags at 15° C and kept at a constant moisture level (field moisture). In the case of the arable site soil samples were transferred into plastic pots without lids and kept in the incubator at constant temperature (30° C) and water content (field moisture). The procedure of measuring $\delta^{13}\text{CO}_2$ was the same as for samples from the other sites.

2.2.3. Isotopic measurements

C isotope ratios of the CO₂ in the air samples from soil incubations were measured either by a tunable diode laser, TDL (Sites 1, 2 and 3 i.e. Kānuka stand, Montane grassland

and Arable), or by isotope ratio mass spectrometer (Site 4, Peatland). Gas samples were drawn directly from Tedlar bags into a TDL (TGA100A; Campbell Scientific Inc., Logan UT, USA). The TDL measures concentrations of the CO₂ isotopologues, and these were converted to delta notation ($\delta^{13}\text{C}_{\text{V-PDB}}$, in ‰) as described by Barbour *et al.* (2007) where:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{V-PDB}}} - 1 \right) * 1000 \quad (2.1)$$

with the R measured as the $^{13}\text{C}/^{12}\text{C}$ of the sample or V-PDB. Typical precision for isotope analysis was ± 0.02 ‰ (± 1 standard error (SE), $n = 45$). When using a mass spectrometer to measure C isotope ratios, gas samples were sub-sampled from the Tedlar bags by attaching a syringe needle to the outlet of the bag, opening the valve while pressing the bag gently to flush the needle, before inserting a pre-evacuated 10 ml septum-capped vial onto the needle (Midwood *et al.*, 2006). Three replicate samples were taken from each bag and then analysed using an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus^{XP}) interfaced to a Gas Bench II and PAL autosampler (Thermo Fisher, Bremen, Germany); typical precision for isotope analysis of compressed air was ± 0.03 (± 1 SE, $n = 28$). The $\delta^{13}\text{C}$ of the solid soil material was measured using a continuous flow isotope ratio mass spectrometer (Thermo Finnigan Delta Plus^{advantage}) interfaced to an elemental analyser (Thermo FlashEA1112, Thermo Fisher, Bremen, Germany).

2.2.4. Chemical extractions

Additional replicate 50 mm diameter soil cores were taken to a depth of 250 mm under all treatments from all sites, but peatland. Soil samples were analysed for hot water extractable C (HWECC). The protocol used for chemical extractions was adapted from the method of Ghani *et al.* (2003). Soil samples were air-dried and sieved through a 2- mm sieve. Subsamples of 3 g were weighed into centrifuge tubes, 30 cm³ of deionised (DI) water was added, and tubes were capped and tumbled on the end-over-end shaker for 30 min. Tubes were then centrifuged for 20 min at 3500 rpm and the supernatant filtered through a pre-

leached filter paper into vials for DOC analysis (results not shown). The mass of the tube with the soil and the remaining water was recorded and the tubes were refrigerated until HWEC analyses were performed. Another 30 cm³ of DI water was added to the same tubes, and the tubes were capped and shaken thoroughly to re-suspend the soil samples in the water. The tubes were placed in a hot water bath at 80° C for 16 hours, and then centrifuged for 20 min at 3500 rpm. The supernatant was filtered into vials for HWEC analysis. DOC and HWEC were measured on a Shimadzu TOC - 5000A analyser (Tokyo, Japan).

2.2.5. Allophane addition experiment

A sample of allophane was prepared by sampling the 2Bw₃ horizon (45 - 100 cm) of the New Plymouth brown loam – Typic Orthic Allophanic Soil (Hewitt, 2010) and heating in a muffle oven at 500° C for 4 hours (Blakemore *et al.*, 1987) to oxidise all organic matter present. This left only the active mineral content: allophane and other active clay minerals and iron oxides capable of binding labile SOM. This process was repeated with a sample of silica sand, which was used as a control treatment.

Three replicate 50 mm diameter soil cores were taken to a depth of 250 mm from the permanent pasture plots from the arable site which had not been cultivated for at least 25 years prior to sampling. As previously, each core was broken open and visible roots and any stones quickly removed by hand and discarded. Soil samples were subsampled for moisture content analysis (by drying at 105°C to a constant weight). The soil sample was put in a plastic pot and weighed. Allophane or sand (n = 3) was added in proportion equivalent to 50 % of dry mass of soil, the mixture was put in a Tedlar[®] bag, sealed and thoroughly mixed by shaking. The soil sample remained in the bag for 2.5 h, after which the bag was evacuated and flushed 4 - 5 times with CO₂-free air as described above. Samples were incubated *in situ* at an ambient temperature (about 20°C) to allow newly respired CO₂ to accumulate. Aliquots

of gas were regularly removed to check the CO₂ concentration. After 3 h, a gas sample was collected and the $\delta^{13}\text{CO}_2$ measured using the TDL as described above.

Soil mixtures were transferred into plastic pots without lids and incubated at a constant temperature (30° C) for two weeks. Every 1-3 d respired $\delta^{13}\text{CO}_2$ were analysed by transferring soil mixtures into Tedlar bags, using the protocols described above. After 14 d of incubation soil mixtures were sieved through a 2- mm sieve and analysed for hot water extractable C using the protocol adapted from the method by Ghani *et al.* (2003). Pure sand and allophane were also analysed.

Sigma Plot (v 8.0) was used to fit exponential decay functions

$$\delta^{13}\text{CO}_2 = y_0 + a * \exp^{-b * t} \quad (2.2)$$

to the time sequence of each replicate soil core short-term incubation; where y_0 is equilibrium $\delta^{13}\text{CO}_2$ value in the short-term incubation, t is duration of incubation and a and b are the coefficients to characterise the exponential decay function. In R (v. 2.11.1), linear models were fitted to assess the site effect on the resulting coefficients (y_0 , a and b , separately) and HWEC. A post-hoc Tukey test was performed ($\alpha = 0.05$) to test for significantly different coefficient values between sites.

2.3. Results

2.3.1. Short-term soil incubations

Soil-respired $\delta^{13}\text{CO}_2$ for all sites showed a similar trend through time: initial values of $\delta^{13}\text{CO}_2$ (y_{start}) within a few minutes of the soil being sampled were enriched (in the range -22.5 to -23.9‰, **Figure 2.1**) relative to the $\delta^{13}\text{C}$ signature of the solid, whole soil (**Table 2.2**). The values for the montane grassland and the arable sites were statistically more enriched in ^{13}C ($P < 0.05$) compared to the peatland (**Table 2.2**). During the subsequent incubation $\delta^{13}\text{CO}_2$ became more depleted and stabilised at an equilibrium value relatively depleted in ^{13}C (in the range -25.8 to -27.5‰) after 50 - 350 min, depending on the site (**Figure 2.1**). For the kānuka stand, montane grassland and the arable site this was depleted compared to the $\delta^{13}\text{C}$ signature of the solid, whole soil and for the peatland site still slightly enriched (**Table 2.2**). The equilibrium values (y_0 for the fitted functions) for the montane grassland and peatland were significantly more enriched in ^{13}C ($P < 0.05$) compared to the kānuka stand and the arable sites (**Table 2.2**). The slope of the curve, which indicates the speed of the change in $\delta^{13}\text{CO}_2$, was different for the peatland site compared to the arable site, showing that the soil from the latter site was the fastest to respond, and the isotopic values stabilised 50 - 60 min after the disturbance. The soil from the peatland site was the slowest to reach equilibrium, as 330 min were needed for the values to stabilise. To check that the rapid change in $\delta^{13}\text{CO}_2$ during incubations was not due to root respiration, samples of the soil from the kānuka stand were sieved to remove all roots prior to incubation. The value of $\delta^{13}\text{CO}_2$ was then measured by incubation 100 min after each core had been taken, giving a value of -27.9 ± 0.20 ‰ ($n=3$). This value was slightly depleted compared to the incubations without sieving (-27.0 ± 0.14 ‰, **Table 2.2**).

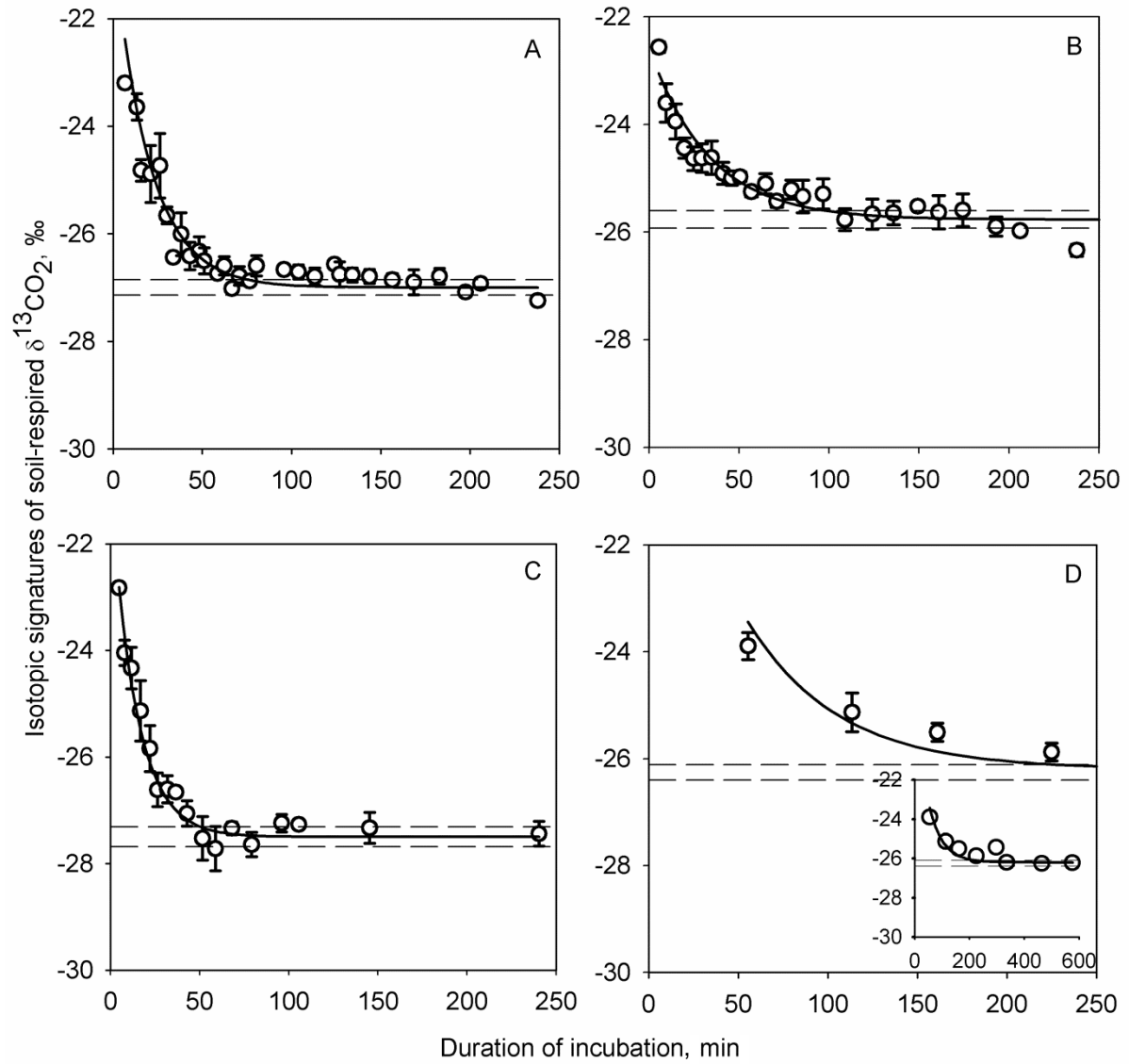


Figure 2.1. Isotopic composition of soil-respired $\delta^{13}\text{CO}_2$ measured in short-term incubations (mean \pm standard error) and exponential decay functions fitted: (A) Kānuka stand $n = 5$; (B) Montane grassland (undisturbed treatment) $n = 4$; (C) Arable (no tillage treatment) $n = 3$; (D) Peatland $n = 5$, small insert shows the full incubation curve. Dashed lines indicate equilibrium values of $\delta^{13}\text{CO}_2$: $y_0 \pm$ standard error.

2.3.1. Long-term soil incubations

Through time, $\delta^{13}\text{CO}_2$ of respired CO_2 returned to similar to the initial relatively enriched starting values at 3 out of 4 sites (**Figure 2.2**). The timing of this trend depended on the site – only 20 - 80 d were needed for $\delta^{13}\text{CO}_2$ values from the montane grassland and 300 d for kānuka stand and peatland to revert back to the starting (enriched in ^{13}C) values.

Large scale soil perturbation had a significant effect on the time needed for $\delta^{13}\text{CO}_2$ to change in long-term incubations (**Figure 2.2B**). Comparison of the change in $\delta^{13}\text{CO}_2$ through time at the montane grassland site showed that the undisturbed soil took four times longer to revert to its original value than the soil that had previously been excavated and mixed again. The results from chemical extractions also showed that the undisturbed soil under native tussock vegetation had 2.3 times the amount of hot water extractable C compared to perturbed soil (**Table 2.3**).

In contrast to the other sites, $\delta^{13}\text{CO}_2$ from the arable site remained depleted in ^{13}C and did not return back to the initial values after 405 d (**Figure 2.2C**). A small enrichment in $\delta^{13}\text{CO}_2$ was observed during the first 12 d of the incubation, but after this initial rise the isotopic values remained relatively depleted in ^{13}C for the rest of the incubation. However, the mean $\delta^{13}\text{CO}_2$ value on day 405 of the incubation was significantly more enriched in ^{13}C compared to day 1 ($P < 0.05$).

Table 2.2. Parameters (y_0 , a , b) for the fitted exponential decay functions using Equation (2.2), measured initial starting values (y_{start}) for the short-term incubations and $\delta^{13}\text{C}$ values of the solid, whole soil.

	Kānuka stand	Montane grassland	Arable	Peatland
y_0 , ‰	-27.00 ^a (0.14)b	-25.77 (0.16)a	-27.50 (0.19)b	-26.26 (0.14)a
a	6.44 (1.63)a	3.20 (1.82)a	6.55 (2.10)a	8.39 (1.63)a
b	0.05(0.01)ab	0.03 (0.01)ab	0.07 (0.01)a	0.02 (0.01)b
y_{start} , ‰	-23.23(0.15)ab	-22.57(0.17)a	-22.88(0.20)a	-23.89 (0.15)b
$\delta^{13}\text{C}$ whole, solid soil, ‰	-26.8 (0.02) Millard et al., 2010	-25.61(0.09)	-26.73 (0.06)	-27.61 (0.03) Artz, pers.comm.

^aMean values with standard errors in brackets. Differences between sites using post-hoc Tukey test ($P < 0.05$) are shown by different lower case letters.

Table 2.3. Total soil C and hot water extractable carbon (HWEC) estimates across sites.

	Kānuka stand	Montane grassland		Arable		Peatland
		Disturbed area	Undisturbed area	No-tillage	Pasture plots	
HWEC, $\mu\text{g C/g soil}$	642 ^a (8)b	856 (109)b	2008 (266)a	704 (5)b	1813 (54)a	No data
Total soil C estimates, %	3.1	3.3	5.1	1.9	2.6	55
Proportion of HWEC of total soil C, %	2.07	2.59	3.94	3.70	6.97	No data

^aMean values with standard errors in brackets. Differences between sites using post-hoc Tukey test ($P < 0.05$) are shown by different lower case letters.

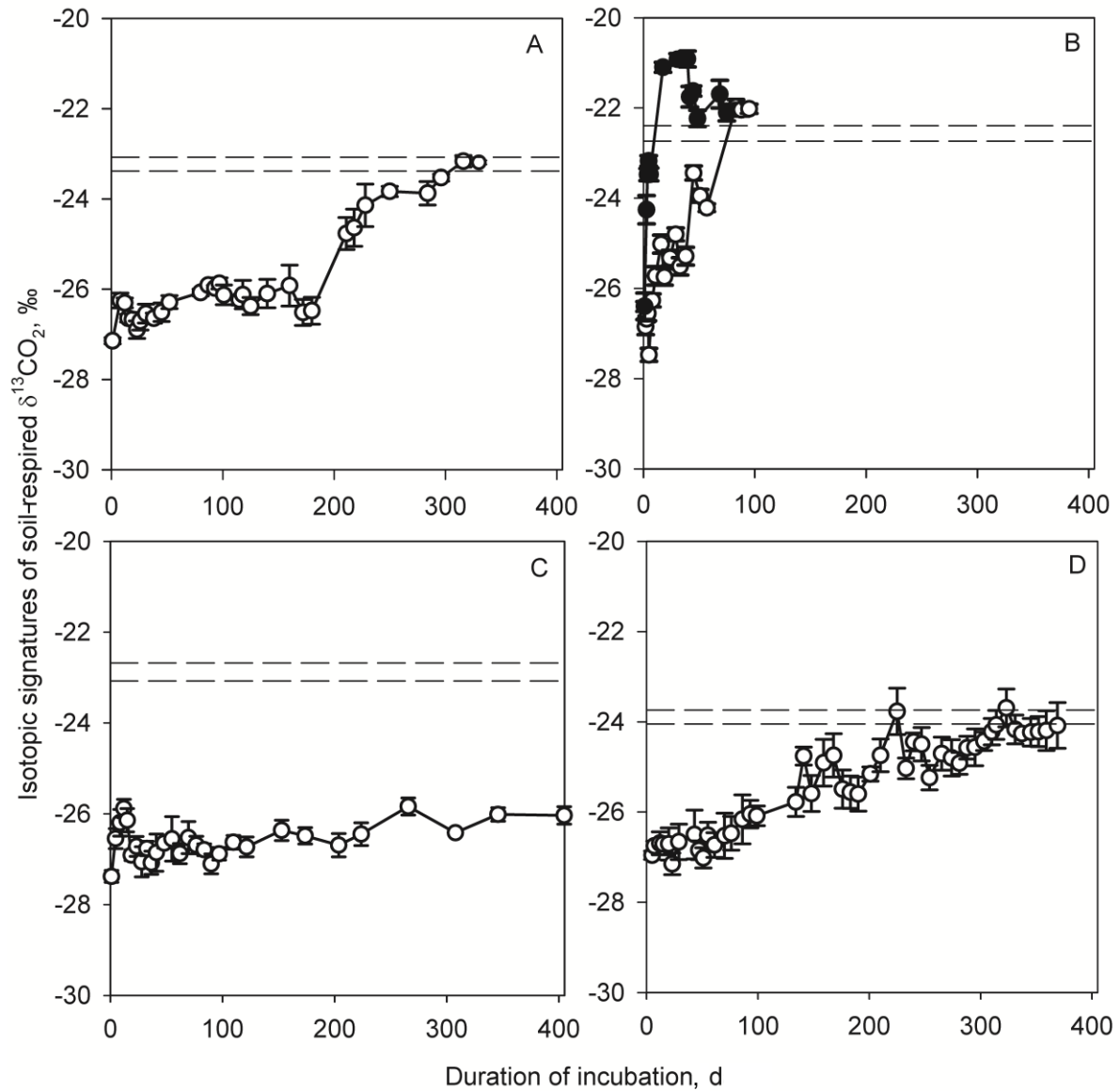


Figure 2.2. Isotopic composition of soil-respired $\delta^{13}\text{CO}_2$ measured in long-term soil incubations (mean \pm standard error): (A) Kānuka stand $n = 5$; (B) Montane grassland: symbols (o) indicate undisturbed soil ($n = 4$) and (●) perturbed soil ($n = 4$); (C) Arable (no tillage treatment) $n = 3$; (D) Peatland $n = 5$. Dashed lines indicate initial starting $\delta^{13}\text{CO}_2$: $y_{\text{start}} \pm$ standard error.

2.3.2. Allophane addition experiment

Soil-respired $\delta^{13}\text{CO}_2$ became more enriched during first 6 d of incubation following allophane addition and stabilised at a value of $-20.43 \pm 0.12 \text{ ‰}$ (**Figure 2.3**). $\delta^{13}\text{CO}_2$ in the two control treatments – soil alone and soil with sand addition – were similar and remained stable and relatively depleted in ^{13}C ($-28.25 \pm 0.03 \text{ ‰}$ and $-28.23 \pm 0.04 \text{ ‰}$, respectively) during the whole incubation. The amount of HWEC from soil after allophane addition ($718 \pm 43 \text{ } \mu\text{g C/g soil}$) was 2.5 times less than the control treatments – soil alone and with sand addition (1813 ± 54 and $1785 \pm 63 \mu\text{g C/g soil}$, respectively). The differences between control treatments were not statistically significant ($P < 0.05$). The amount of HWEC extracted from pure allophane and sand was negligible (less than $5 \text{ } \mu\text{g C/g substrate}$).

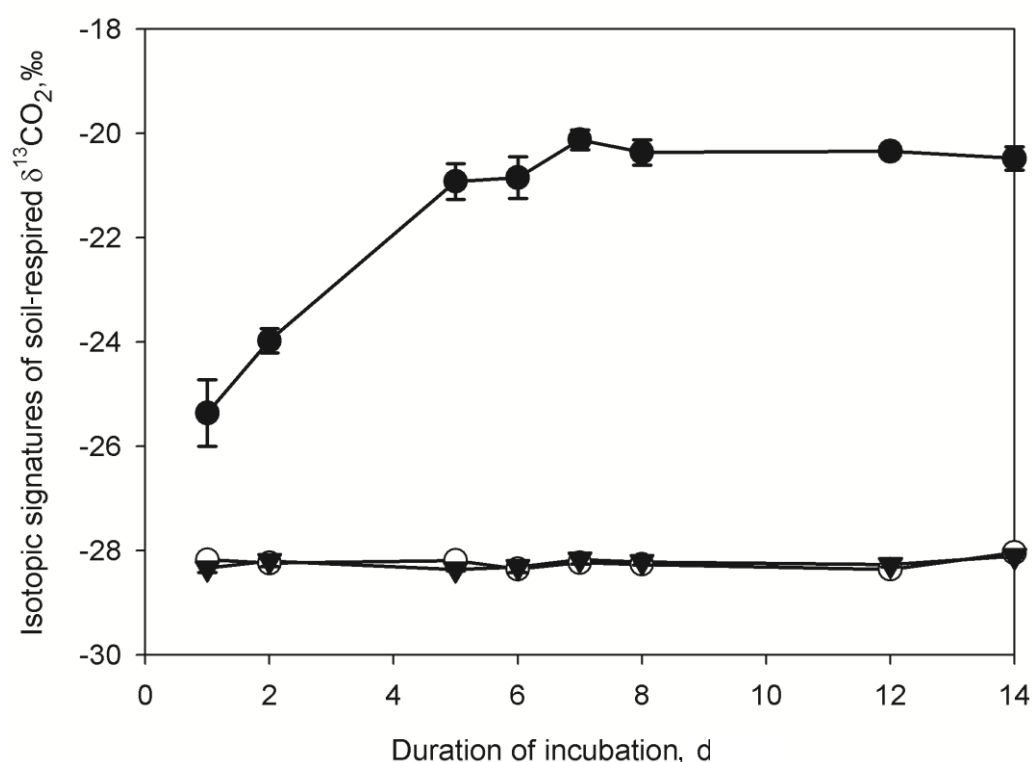


Figure 2.3. Isotopic composition of soil-respired $\delta^{13}\text{CO}_2$ incubated without any additions (▼) and after addition of allophane (●) or silica sand (○) from the control pasture plots from the arable site: mean \pm standard error ($n = 3$). Control pasture plots from the arable site were used as the most reflective of an ‘undisturbed’ agricultural soil as they had not been cultivated for at least 25 years prior to sampling.

2.4. Discussion

The trend in soil-respired $\delta^{13}\text{CO}_2$ seen in the short-term soil incubations was consistent with previously inaccessible, relatively depleted labile C becoming available as a result of soil disturbance, due to sampling and incubating. Long-term incubations (in 3 out of 4 sites) resulted in isotopic signatures reverting back to the initial starting values as labile C pools were exhausted. Our results suggest that the pattern of $\delta^{13}\text{CO}_2$ over time is due to changes in labile C substrate utilisation. This interpretation was confirmed with the results of long-term incubations at a site, which had a large scale soil perturbation treatment, as well as an experimental approach which immobilised labile soil C onto allophane. Below, the contribution of these results to our understanding of the dynamics of labile soil C pools is discussed.

2.4.1. Short-term incubations

A rapid change in $\delta^{13}\text{CO}_2$ with disturbance due to soil core extraction was observed for all sites (**Figure 2.1**) and was similar to the trend described by Millard *et al.* (2010). The rapid depletion of $\delta^{13}\text{CO}_2$ was likely a result of a release of labile C after a soil disturbance which resulted in a change in the physical protection of SOM. At the start of soil incubations, just minutes after the soil sample was taken from the ground, soil-respired $\delta^{13}\text{CO}_2$ was dominated by relatively ^{13}C -enriched SOM, which represents a large (up to 95 % of SOM) pool in many soils. As considered in the CENTURY model, the ‘intermediate’ pool represents 60 - 85% of SOM, the ‘passive’ pool – 10 - 40 %, and the highly active ‘labile’ pool only accounts for 5 % of SOM (Parton *et al.*, 1987; Schimel *et al.*, 1994; Townsend *et al.*, 1997). This initial $\delta^{13}\text{CO}_2$ value (y_{start}), if obtained as quickly as possible after a soil disturbance, was considered to reflect the *in situ* mixture of respiration of the continuum of labile - recalcitrant C pools in an undisturbed soil. When soil was broken up, $\delta^{13}\text{CO}_2$ values became more dominated by newly released labile C.

The presence of fine roots could have potentially affected the observed trend during the short-term incubations as the $\delta^{13}\text{CO}_2$ values of root respiration are more depleted in ^{13}C compared to SOM and soil respiration (Bowling *et al.*, 2008). However, visible roots were quickly but consistently removed from the soil sample before the start of the incubation. Fine roots left in the soil would tend to die quickly and thus be unlikely to have a great affect upon $\delta^{13}\text{CO}_2$. When soil from the kānuka stand was sieved to remove roots, the resulting $\delta^{13}\text{CO}_2$ was slightly depleted compared to un-sieved soil, probably due to the greater degree of disturbance caused by sieving the soil. Moreover, the observed shift in $\delta^{13}\text{CO}_2$ during our short-term incubations was coupled with a rapid increase in the rate of respiration (which was not quantified but was evident from the CO_2 concentrations in the gas samples analysed at each time point), which is unlikely to have been caused by dying roots. Our results from the long-term incubations show that $\delta^{13}\text{CO}_2$ of the soil-respired CO_2 remained relatively depleted for at least 20 days (and up to 300 days), far longer than active root respiration could have contributed to the CO_2 . Taken together, these results suggest that it was unlikely that residual root respiration contributed very much to the changes in $\delta^{13}\text{CO}_2$ seen during the short-term incubations.

The soil from the peatland site was characterised by a large total C content (55 %, **Table 2.3**), practically all of which was relatively old (approximately 4000 years old – due to mechanical removal (peat harvesting) of the surface layers prior to sampling), recalcitrant C. Evidence for the recalcitrance of the soil C at this site comes from the: (1) low relative proportions of cellulose and other polysaccharides in relation to fats, waxes, lipids, phenolic and other aromatic structures that would require more energy to decompose (Artz *et al.*, 2008a); (2) low microbial biomass, (3) low basal respiration values (Trinder *et al.*, 2008a); (4) extremely low net ecosystem respiration in the growing season and (5) lack of any change with depth in microbial substrate utilisation profiles (Artz *et al.*, 2008b). After sampling the

peat soil, a significantly slower response of $\delta^{13}\text{CO}_2$ was observed than for the other soils, which also resulted in longer time for the $\delta^{13}\text{CO}_2$ to stabilise (**Figure 2.1**) and a more enriched equilibrium $\delta^{13}\text{CO}_2$ value (y_0) relative to the other sites (**Table 2.2**).

Labile SOM often consists of fresh plant residues and is physically protected from microbes within macro- and micro-aggregates due to: (1) compartmentalisation of substrate and microbial biomass, (2) compartmentalisation of microbial biomass and grazers, and (3) reduced oxygen diffusion into macro- and micro-aggregates (Six *et al.*, 2002; von Lützow *et al.*, 2006). Undisturbed soils accumulate labile C due to the enhanced protection of SOM by aggregates (Golchin *et al.*, 1994; Six *et al.*, 2002) and the interaction between soil minerals and SOM. This results in the formation of stable organo-mineral complexes, and ultimately in long-term stabilisation of SOM by both physical and chemical protection of SOM adsorbed on mineral surfaces (Plaza *et al.*, 2013). Significant soil disturbance alleviates the physical protection of labile SOM due to disturbance of soil aggregates (especially macro-aggregates) and oxygen diffusion into the soil. Thus physically-protected, labile SOM becomes available as a respiratory substrate. These changes in substrate utilisation are likely to have determined the shifts in $\delta^{13}\text{CO}_2$ during short-term soil incubations.

It is worth mentioning some of the potential isotopic disequilibrium effects that could have contributed to the observed pattern in $\delta^{13}\text{CO}_2$ during the short-term soil incubations. These effects are known to occur if the soil CO_2 efflux is isotopically different from concurrent CO_2 production due to transient conditions in soil air, which can happen due to diffusion of CO_2 in soil air, advection of soil air, dissolution of CO_2 in soil water or an introduction of an isotopic tracer, such as $^{13}\text{CO}_2$ or $^{14}\text{CO}_2$ (Gamnitzer *et al.*, 2011). In our experiments natural abundance $\delta^{13}\text{C}$ discrimination was used, so no isotopic tracers were applied. Moreover, neither disruption of the diffusion of soil CO_2 nor creation of advective transport of bulk flow could be applied in our case as soil surface measurements with closed

chambers were not made. By our method of bag incubation only currently produced CO₂ was measured. The combination of the short and long-term incubations and the allophane addition experiments all suggest that the changes in $\delta^{13}\text{CO}_2$ observed were not an artefact due to disequilibrium effects.

2.4.2. Long-term incubations

During long-term incubations labile C pools, with a relatively depleted ^{13}C signature, were preferentially utilised by microbes (Crow *et al.*, 2006). However, as the incubation proceeded and the labile C pools were exhausted, $\delta^{13}\text{CO}_2$ returned to more enriched ^{13}C isotopic values which reflected greater use of recalcitrant C (Townsend *et al.*, 1997; Ehleringer *et al.*, 2000; Pendall & King, 2007). So, when disturbed soil samples were incubated for long enough a reversal in isotopic signatures of soil respiration back to initial enriched ^{13}C values was observed (**Figure 2.2A, B, D**: kānuka stand, montane grassland and peatland). Due to slow process rates and a small size of the microbial community (Artz *et al.*, 2008b; Trinder *et al.*, 2008a) over 300 d were needed for the labile C pool in the peat soil to become exhausted and the $\delta^{13}\text{CO}_2$ to revert back to their initial values (**Figure 2.2D**).

However, as found by Schweizer *et al.* (1999) and argued by Crow *et al.* (2006) substrate pool switching from depleted to enriched in ^{13}C values is not the only driving factor of the respired $\delta^{13}\text{CO}_2$. Microbial discrimination against the isotopically heavy ^{13}C in the most labile C pool and preferential use of identical substances with lighter (^{12}C) isotope (Boschker & Middelburg, 2002) might yet be another possible explanation for the transition from isotopically depleted to enriched values of respired $\delta^{13}\text{CO}_2$. Fernandez and Cadisch (2003) found that the degree of isotopic discrimination against ^{13}C was species-specific, but it also depended on decomposition stage (two white rot fungi changed the patterns over the course of the 76-day incubation). Moreover, Satruckova *et al.* (2000) suggested that the growth stage of the microbial population influenced the degree of isotopic discrimination:

growing cells in microorganisms synthesized enriched in ^{13}C compounds while growth-limited cells produced depleted in ^{13}C storage material. However, according to Schweizer *et al.* (1999) microbial discrimination is hard to quantify in a complex environment. Blagodatskaya *et al.* (2011), considered that microbial discrimination plays a less dominant role in the changes of ^{13}C signature of soil pools compared to microbial preferential utilisation of recent (labile) *versus* old (recalcitrant) C. Furthermore, SOM decomposition might not only depend on the substrate biochemistry but also on the ability of the existing microbial community to decompose the available substrate (Curiel Yuste *et al.*, 2007). More labile C, that is readily decomposable and available at the beginning of the incubation, favours opportunistic/cheaters (r-strategic) over enzyme producers (K-strategic); whereas depletion of the labile C in the later stages of decomposition favours enzyme producers that are able to break down more stable fractions of SOM (Allison, 2005; Curiel Yuste *et al.*, 2007). These alterations in microbial community structure may occur on the scale of hours/days depending on the substrate availability and enzyme diffusion (Allison, 2005). They may contribute to shorter term shifts as well as the change from depleted to enriched respired $\delta^{13}\text{CO}_2$ during the later stages of the incubations after the consumption of easily available labile C pools as suggested by Crow *et al.* (2006).

Labile SOM is considered to be the most vulnerable to loss after a major soil perturbation (Six *et al.*, 2002). It took four times longer for $\delta^{13}\text{CO}_2$ to revert back to the starting values in undisturbed soil compared to the soil that experienced previous large scale perturbation (**Figure 2.2B**). As labile C tends to be more depleted in ^{13}C than recalcitrant C, this suggests that the change in $\delta^{13}\text{CO}_2$ through time was due to labile C pools being exhausted, with a smaller labile pool size in heavily perturbed soil. This hypothesis was supported by the amount of HWEC in soils, which is an independent measure known to be an early indication of organic matter loss (Ghani *et al.*, 2003).

In contrast, $\delta^{13}\text{CO}_2$ from the arable site remained depleted in ^{13}C and did not return back to the starting value after 405 d (**Figure 2.2C**). As far as I am aware only Pendall and King (2007), who carried out long-term incubations of top- and sub-soils from a shortgrass steppe, observed $\delta^{13}\text{CO}_2$ becoming more depleted in ^{13}C during the final phase of incubations. The authors interpreted this as resulting from lignin utilisation – a compound that is much harder for microbes to metabolise due to its aromatic ring structure compared to sugars and starch. $\delta^{13}\text{C}$ values for sugars and starch are 1 - 2 ‰ enriched whereas $\delta^{13}\text{C}$ values for lignin are 2 - 4 ‰ depleted in ^{13}C compared to values from bulk leaves (Fernandez *et al.*, 2003; Bowling *et al.*, 2008). A small enrichment in $\delta^{13}\text{CO}_2$ noticed at the very start of the incubation (**Figure 2.2C**) was probably due to microbial utilisation of easily available labile soil C over the first 12 d of incubation. A similar trend has been reported before (Crow *et al.*, 2006) and is in agreement with the long-term incubations from the other sites studied (**Figure 2.2A, B, D**). Plante and McGill (2002) even observed a similar timing for the rise in $\delta^{13}\text{CO}_2$ - approximately two weeks after the start of the incubation. However, our observation that, after an initial enrichment, the isotopic values remained relatively depleted in ^{13}C for the rest of the incubation is a curious finding. A better understanding of the factors influencing the dynamics of labile C pools is necessary to be able to explain the trend observed.

2.4.3. Allophane addition experiment

To test if the changes in isotopic signatures of soil-respired $\delta^{13}\text{CO}_2$ described above were due to changes in labile C substrate utilisation following soil disturbance, an experimental approach which immobilised labile soil C onto allophane was used. Parfitt *et al.* (1999) showed that organic compounds can be bound onto allophane through ligand exchange between carboxyl as well as carbohydrate groups of organic matter and the hydroxyl groups bonded to structural aluminium in allophane. For this manipulation soils from the permanent pasture plots from the arable site which had not been cultivated for at least 25 years prior to sampling were used (Thomas *et al.*, 2008; Baldock *et al.*, 2010). This maximised the chance of having relatively large labile soil C pools. As expected, $\delta^{13}\text{CO}_2$ became more enriched in ^{13}C during the first several days of soil incubation after the allophane addition (**Figure 2.3**), indicating that the labile SOM pool, with a depleted $\delta^{13}\text{CO}_2$ signature, was bound to allophane forming Al-organic complexes (Baldock & Skjemstad, 2000; Yuan *et al.*, 2000). As the labile C became unavailable, $\delta^{13}\text{CO}_2$ became progressively more dominated by the respiration of recalcitrant SOM with an enriched $\delta^{13}\text{CO}_2$ signature. In samples amended with sand, which didn't react with SOM, and in those soil samples without any additions, $\delta^{13}\text{CO}_2$ remained depleted in ^{13}C , representing the respiration of labile C pools. Saggar *et al.* (1994) observed that the turnover of carbohydrate C was retarded in allophanic soils and suggested stabilisation by allophane of both microbial biomass and their substrates as a possible explanation. The hypothesis that shifts in $\delta^{13}\text{CO}_2$ over the course of soil incubations were due to changes in labile C substrate utilisation was also supported by the results of the chemical extractions – the amount of hot water extractable C decreased significantly after allophane addition, showing that labile C was bound onto allophane and became unavailable because of the chemical stabilisation mechanism.

2.5. Summary

In this study, strong temporal trends in $\delta^{13}\text{CO}_2$ were observed during short- and long-term soil incubations following a soil disturbance. These results suggest that shifts in $\delta^{13}\text{CO}_2$ over the course of soil incubations were due to changes in labile C substrate utilisation. This interpretation was confirmed with the results of a disturbance treatment as well as an experimental approach which immobilised labile soil C onto allophane. The results show that the isotopic analysis of soil-respired CO_2 can be a powerful technique. However, further studies combining soil incubations with the measurements of $\delta^{13}\text{CO}_2$ signatures and respiration rates are needed to fully quantify the consequences of disturbance on the labile component of soil C.

**CHAPTER 3: FACTORS CONTROLLING LABILE SOIL
ORGANIC MATTER VULNERABILITY TO LOSS
FOLLOWING DISTURBANCE ASSESSED BY
MEASUREMENT OF SOIL-RESPIRED $\delta^{13}\text{CO}_2$**

3.1. Introduction

Labile soil organic matter (SOM) is comprised of easily decomposable compounds that are often characterised by fast decomposition rates and short turnover times (Townsend *et al.*, 1997; Krull *et al.*, 2003). However, the vulnerability of labile SOM to microbial degradation depends on the extent it is protected from microbes (Dungait *et al.*, 2012), through its bonding to mineral surfaces (i.e. forming mineral complexes) and physical protection within soil aggregates (Krull *et al.*, 2003). Several factors can contribute to SOM protection, including reduced oxygen diffusion into soil aggregates and physical separation from soil microbes (Six *et al.*, 2002). SOM, especially its labile component, is regarded as the primary source of nutrients in soils; hence to maintain soil productivity, it is critical to understand the response of labile SOM to environmental change and land management practices.

Various chemical and physical fractionation procedures are widely used to separate and measure the amount of labile SOM (see review by von Lützow *et al.* (2007). Hot water extractable C (HWE) is a common measure of labile SOM, based on the assumption that SOM that is easily degradable by microbial enzymes is more soluble in hot water than other organic fractions in the soil (McLauchlan & Hobbie, 2004). HWE is one of the most sensitive indicators to reflect changes in SOM due to management practices (Ghani *et al.*, 2003). However, as a chemically extracted SOM pool, HWE does not take into account spatial arrangements of organo-mineral complexes and hence physical protection of SOM. Physical protection and microbial accessibility appear to be more important than pure molecular recalcitrance in regulating SOM turnover (Dungait *et al.*, 2012).

The bioavailability of SOM is captured by physical fractionation methods as they are based on the concept that SOM fractions associated with different size particles (e.g., sand, silt and clay) have different structures and functions (Christensen, 2001). Sand-associated

SOM, also known as particulate organic matter (POM), represents only a small proportion (often <10%) of total SOM (Cambardella & Elliott, 1992; Christensen, 2001). Since C from POM is rapidly mineralised and is usually the first to be affected by changes in management practices, it is widely regarded as a labile fraction (Balesdent, 1996). Silt and especially clay particles provide reactive surfaces capable of binding labile substrates and protecting them through physicochemical stabilisation mechanisms (Six *et al.*, 2002; Krull *et al.*, 2003). A specific physicochemical property – soil surface area (SSA), which is positively correlated with SOM content and C content of soil fractions (Kahle *et al.*, 2002; Wiseman & Püttmann, 2005), may indicate SOM protection capacity and its bioavailability in soils.

An alternative to the fractionation of SOM for measuring the labile component might be the use of ^{13}C signatures of soil-respired CO_2 . Millard *et al.* (2010) found a rapid depletion of respired $\delta^{13}\text{CO}_2$ from a forest soil within a few hours of incubation, which they ascribed to disturbance (as a result of extracting soil cores and removal of roots), causing small but relatively labile SOM pools, previously protected from microbial activity, to become available as respiratory substrates. Zakharova *et al.* (2014) confirmed that shifts in soil-respired $\delta^{13}\text{CO}_2$ over the course of soil incubations reflected changes in labile substrate utilisation. They found a rapid depletion of $\delta^{13}\text{CO}_2$ during the first hours following soil sampling indicating increased availability of labile SOM, while a subsequent reversion back to the initial relatively enriched starting values after several months was due to labile SOM pools becoming exhausted (Zakharova *et al.*, 2014).

Here, the hypothesis, that during short-term soil incubations, equilibrium soil-respired $\delta^{13}\text{CO}_2$ is a direct function of the amount of labile SOM and soil physical conditions, is tested. Soils were incubated over several hours and soil properties (such as total soil C, HWEC, SSA, sand, silt and clay content and C content of soil fractions) and changes in soil-respired $\delta^{13}\text{CO}_2$ were measured. Models were fitted to the relationships between equilibrium

soil-respired $\delta^{13}\text{CO}_2$ and soil properties to identify which combination of properties best explained the observed equilibrium soil-respired $\delta^{13}\text{CO}_2$. Additionally, to determine in more detail the role of soil surface area in determining labile SOM availability and protection from loss, soil was incubated with allophane – a clay mineral with a large, active surface area.

3.2. Materials and methods

3.2.1. Soils

Three replicate paddocks with permanent, irrigated pasture sown with perennial ryegrass (*Lolium perenne* L.) and clover (*Trifolium repens* L.) were selected on each of three agricultural soils, which were classified as Lismore, Temuka and Wakanui soils (New Zealand soil classification, Hewitt, 2010) and Typic Dystrustept, Typic Endoaquept, and Aquic Haplustept, respectively, (USDA soil classification, Soil survey staff, 2010) on the Canterbury plains, in the South Island of New Zealand. Three replicated soil cores were taken from each pasture paddock at each site. Site descriptions and soil textural properties are summarised in **Table 3.1**.

Table 3.1. Site descriptions and soil textural properties

Site	Soil	Latitude, longitude	Sand, ^a mg g ⁻¹ soil	Coarse silt, ^a mg g ⁻¹ soil	Fine silt, ^a mg g ⁻¹ soil	Clay, ^a mg g ⁻¹ soil
1 ^b	Lismore	43° 53' 05" S 172° 00' 59" E	151 ^c (8.1)	509 (2.3)	202 (2.9)	327 (2.8)
2	Lismore	43° 38' 27" S 171° 53' 08" E	133 (6.9)	616 (63.2)	227 (4.0)	357 (2.2)
3	Lismore	43° 47' 39" S 171° 34' 54" E	308 (8.5)	436 (54.5)	170 (2.8)	345 (9.9)
4 ^b	Temuka	43° 47' 34" S 172° 14' 43" E	42 (2.5)	574 (40.1)	321 (1.7)	453 (5.4)
5	Temuka	43° 46' 38" S 172° 17' 48" E	135 (12.9)	464 (69.5)	210 (3.2)	525 (26.4)
6	Temuka	43° 39' 40" S 172° 27' 04" E	176 (4.9)	319 (45.1)	180 (3.4)	440 (5.3)
7 ^b	Wakanui	43° 40' 03" S 172° 28' 11" E	349 (25.3)	481 (11.7)	164 (10.2)	271 (18.4)
8	Wakanui	43° 39' 45" S 172° 19' 11" E	121 (13.4)	405 (11.7)	250 (16.1)	391 (13.0)
9	Wakanui	43° 39' 44" S 172° 18' 58" E	181 (10.1)	346 (20.7)	212 (4.6)	362 (4.4)

^aParticle sizes: sand > 53 µm, coarse silt 53-20 µm, fine silt 20-5 µm, and clay <5 µm.

^bSoils from sites 1, 4 and 7 were used for the allophane addition experiment.

^cMean values (± 1 standard error, n=3).

3.2.2. Soil sampling and incubations

The protocol used for soil sampling followed Millard *et al.* (2010). Soil cores (50 mm diameter) were taken to a depth of 150 mm, broken open and visible roots and any stones quickly (within minutes) removed by hand and discarded. The remaining soil from each core was mixed and placed in a Tedlar[®] bag (Keika Ventures, Chapel Hill, NC, USA). The bag was sealed and quickly flushed (typically five or six times) with CO₂-free air, until less than 20 ppm CO₂ remained in the bag. Samples were incubated *in situ* at ambient temperature and aliquots of gas were regularly removed to check the CO₂ concentration with a portable infrared gas analyser IRGA EGM-4 (PP Systems, Hitchin UK). Once a minimum of 350 ppm CO₂ (needed for the isotopic measurements) had accumulated (typically after seven – ten minutes), the headspace gas was transferred to a second Tedlar bag that had previously been flushed twice with CO₂-free air, and then analysed for $\delta^{13}\text{CO}_2$ as described below. The bag with the soil was then flushed with CO₂-free air again and re-incubated until sufficient CO₂ had accumulated for a second sample. This was repeated until $\delta^{13}\text{CO}_2$ had stabilised.

3.2.3. Isotopic measurements

The C isotope ratios of the CO₂ in the bagged air samples from soil incubations were measured by a tunable diode laser, TDL (TGA100A; Campbell Scientific Inc., Logan UT, USA). Gas samples were sucked directly from Tedlar bags into a TDL. The TDL measured concentrations of the three CO₂ isotopologues at 1Hz, namely ¹²C¹⁶O₂, ¹³C¹⁶O₂ and ¹²C¹⁶O¹⁸O (Bowling *et al.*, 2003), and these were converted to delta notation ($\delta^{13}\text{C}_{\text{V-PDB}}$, in ‰) using Equation (2.1). Typical precision for isotope analysis was ± 0.02 ‰ (± 1 standard error (SE), n = 45).

3.2.4. Chemical extractions

The soil samples were taken back to the laboratory and analysed for HWE. The protocol used for chemical extractions was adapted from the method of Ghani *et al.* (2003).

Soil samples were air-dried at 30°C for 24 hours and sieved through a 2 mm sieve. Subsamples (3 g) were weighed into centrifuge tubes, 30 ml of deionised (DI) water added, and tubes capped and shaken for 30 minutes. Tubes were then centrifuged for 20 minutes at 3500 rpm and the supernatant was discarded to remove readily soluble C following the protocol by Ghani *et al.* (2003). The tubes with the soil and the remaining water were weighed. Another 30 ml of DI water was added to the same tubes, and the tubes were capped and shaken to re-suspend the soil. The tubes were placed in a hot water bath at 80°C for 16 hours and then centrifuged for 20 minutes at 3500 rpm. The supernatant was filtered through Advantec 5C filter paper into vials for HWEC analysis. HWEC were measured on a Shimadzu TOC - 5000A analyser (Tokyo, Japan).

3.2.5. Specific soil surface area

The specific soil surface area (SSA in m² g⁻¹) was calculated from the water content of air-dried soil samples using the linear regression reported by Parfitt *et al.* (2001):

$$SSA = 2 * W_A \quad (3.1)$$

where W_A was the water content of air-dried soil samples (g kg⁻¹), calculated as the weight difference between the air-dried sample (30°C for 24 hours) and the subsequently oven-dried sample (105°C for 24 hours to a constant mass) as described by Parfitt *et al.* (2001).

3.2.6. Physical fractionation

The protocol used was adapted from the particle size analysis method described by Gee and Bauder (1986). Air-dried soil (20 g; < 2 mm) was weighed into a glass beaker and DI water (60 ml) added. An ultrasonic probe Bandelin SONOPULS (HD 2200, mean power output 63.6 W) was used to disperse particles in a 60 s treatment, before washing the suspension through a 53 µm sieve using a jet of DI water. The sand fraction retained on the sieve, was dried (60°C) and set aside for C analysis. The <50 µm material was further

separated into 50-20 (coarse silt), 20-5 (fine silt), and <5 μm (clay) fractions by gravity sedimentation, based on particle settling velocities as defined by Stoke's law (Gee & Bauder, 1986). After allowing the silt fractions to settle out, the supernatant water was siphoned off. A minimal amount of solid CaCl_2 was added to flocculate the clay fraction (2 g $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ /litre of suspension) and, after flocculation, the supernatant water was siphoned off. All soil fractions were dried in the oven (60° C), weighed and ground for total C analysis on the LECO TruMac CN analyser (USA) based on the Dumas method (Leco Corporation, 2003) with combustion temperature of 1250° C.

3.2.7. Allophane addition experiment

A sample of allophane was obtained by sampling the Bw horizon (45 - 100 cm) of a New Plymouth brown loam – Typic Orthic Allophanic Soil (Hewitt, 2010). This soil was heated in a muffle oven at 500°C for four hours (Blakemore *et al.*, 1987) to oxidise all organic matter present. The soil residue was comprised mainly of allophane, but also contained other clay minerals and iron oxides capable of binding labile SOM. Allophane is a group of clay-size minerals with short-range order – local clusters of silica, alumina and water in chemical combination – with large active surface area (700 – 1500 $\text{m}^2 \text{g}^{-1}$) (Parfitt, 1990), largely derived from parent materials of volcanic origin. A sample of silica sand, which was heat treated as described above, was used as a control.

The effect of allophane addition on respired $\delta^{13}\text{CO}_2$ from the different soils (Lismore, Temuka and Wakanui) was assessed. A subset of three sites (1, 4, 7) were selected, each a different soil. Nine replicate 50 mm diameter soil cores of each soil were taken to a depth of 150 mm from each site. Each core was broken open and visible roots and any stones quickly removed by hand and discarded as before. Soil samples were subsampled to measure moisture content (by drying in the oven at 105°C to a constant weight). Field moist soil samples were weighed and allophane or silica sand ($n = 3$) was then added as 50% of dry soil

mass; the mixtures were placed in a Tedlar[®] bag, sealed and thoroughly mixed by shaking. The other three soil cores remained untreated and represented the no additions treatment. All soils remained in the bag for 2.5 hours, after which it was evacuated, flushed, incubated and gas samples collected and measured for $\delta^{13}\text{CO}_2$ using the TDL as described above.

Soils were then transferred into plastic pots without lids, weighed and incubated at constant temperature (30°C) and moisture content (field moisture) for two weeks. Every 1 – 3 days, respired $\delta^{13}\text{CO}_2$ was analysed by transferring the soil mixtures into Tedlar bags, using the protocols described above. After 14 days of incubation, the soils were sieved (<2 mm) and analysed for HWEC using the protocol described above. HWEC of the heat-treated sand and allophane were also analysed.

3.2.8. Statistical analysis

Exponential decay functions were fitted to the time sequence of the short-term incubation for each soil core, using Equation (2.2), where y_0 represents the equilibrium $\delta^{13}\text{CO}_2$ value in the short-term incubation duration t , and a and b characterise the shape of the exponential decay function. Data were grouped by replicate soil cores within each site to account for site as a random effect. The models with the different and the same coefficients for Lismore, Temuka and Wakanui soils were compared using Akaike's information criterion (AIC).

Possible relationships between equilibrium values (y_0) of soil-respired $\delta^{13}\text{CO}_2$ and soil properties were analysed using linear mixed-effects models with site and soil classification as random intercepts. A candidate set of models was established to identify which of the soil properties and their combinations (such as HWEC, C content of particle-size fractions, SSA and total soil C content) explained the majority of the variability in the equilibrium isotopic

values. These models reflected plausible hypotheses about the relationships between equilibrium soil-respired $\delta^{13}\text{CO}_2$, the amount of labile SOM and specific soil properties.

The candidate model set was built on the following hypotheses:

1. Total soil C increases the amount of labile SOM.
2. Interactions of HWEC and POM with total soil C give a better estimate of the amount of labile SOM than each of these soil properties on their own.
3. Clay content and SSA reduce the amount of labile SOM that is available and thus vulnerable to loss due to physicochemical stabilisation – binding of SOM onto clay minerals with large, active surface area.
4. Combination of HWEC or POM, total soil C and SOM protection capacity (SSA or clay content) provides the best explanation of equilibrium soil-respired $\delta^{13}\text{CO}_2$, as both the amount of labile SOM and its availability are taken into account.

The models were ranked using the Akaike's information criterion corrected for small sample sizes (AICc) to determine the Kullback-Leibler (KL) best model (Burnham & Anderson, 2002). The AICc identifies the model(s) most strongly supported by the data based on bias-corrected, maximized log-likelihood (LogLik) of the fitted-model with a penalty for the number of parameters used. The model with the smallest AICc (AICc_{\min}) is the most strongly supported. ΔAICc is calculated for each model i as $\Delta i = \text{AICc}_i - \text{AICc}_{\min}$. As a rule of thumb, models with Δi of < 2 also receive substantial support, models with $2 < \Delta i < 7$ receive considerably less support, whereas models with $\Delta i > 8-14$ receive no support (Anderson, 2008). A measure of the strength of evidence for either model (altitudinal trend or not) is described by the model probability (Akaike weights, w_i). This is the probability that model i is the KL best model, given the data and candidate set of models (Anderson, 2008). The sum of w_i of the models in a candidate set equals 1. All analyses were performed in R

(version 3.0.2, R Development Core Team, 2013) and included use of the packages *lme* and *nlme* (Pinheiro *et al.*, 2013).

3.3. Results

3.3.1. Short-term soil incubations

Soil-respired $\delta^{13}\text{CO}_2$ for all soils showed a similar trend through time: initial values of $\delta^{13}\text{CO}_2$ within a few minutes of the soil being sampled started in the range -19.9 to -22.3‰ (**Figure 3.1**). During the subsequent incubation, soil-respired CO_2 became more depleted in ^{13}C and stabilised after 70 - 90 minutes at an equilibrium value (y_0), which was in the range -26.5 to -27.7‰, depending on the soil, (**Figure 3.2A**). The best exponential decay model included fixed a and b coefficients, while equilibrium y_0 values differed between Lismore, Temuka and Wakanui soils. These equilibrium soil-respired $\delta^{13}\text{CO}_2$ values for the Wakanui soil were more depleted in ^{13}C compared to Temuka and Lismore soils (**Figure 3.2A**).

3.3.2. Relationships between $\delta^{13}\text{CO}_2$ and soil properties

Total soil C content and SSA were similar across the soils (**Table 3.2** and **Figure 3.2C, D**). There was less HWE C in the Lismore soil than in Temuka and Wakanui soils (**Figure 3.2B**). C content of the fractions ranged across the analysed soils. The majority of C was associated with the clay fraction, while coarse silt fraction stored very little C (**Table 3.2**).

The model from the candidate set which provided the best fit to the equilibrium soil-respired $\delta^{13}\text{CO}_2$ was a three-way-interaction model between HWE C, total soil C and SSA (**Table 3.3**, $R^2 = 0.87$), which yielded the relationship:

$$y_0 = 108.0 - 2.56 * \text{HWE C} - 3.07 * \text{Soil C} - 3.18 * \text{SSA} + 0.058 * \text{HWE C} * \text{Soil C} + 0.059 * \text{HWE C} * \text{SSA} + 0.074 * \text{Soil C} * \text{SSA} - 0.001 * \text{HWE C} * \text{Soil C} * \text{SSA}.$$

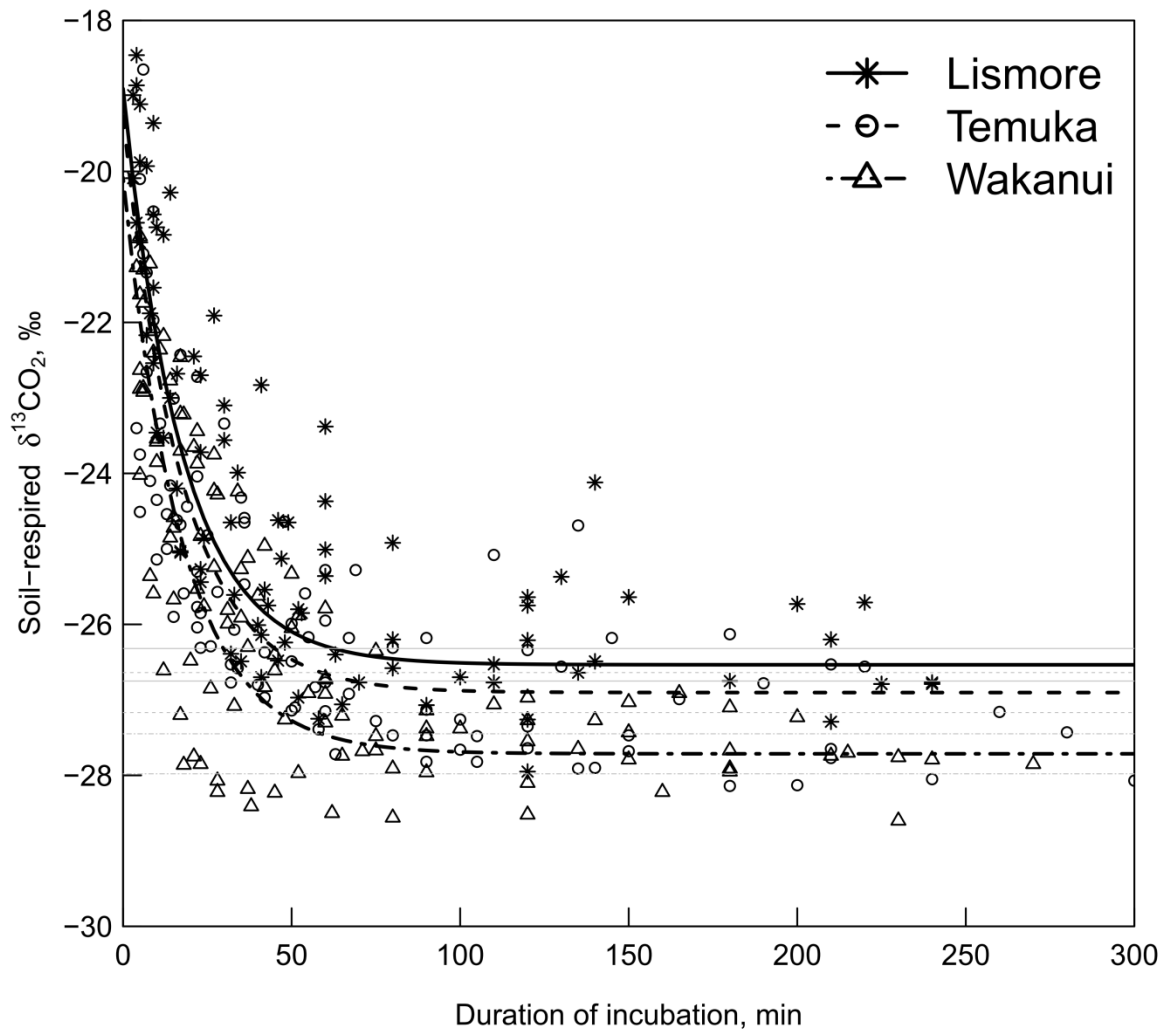


Figure 3.1. Isotopic composition of soil-respired CO₂ measured in short-term incubations on pastures with exponential decay functions fitted: (*) Lismore soil, n=3; (o) Temuka soil, n=3; (Δ) Wakanui soil, n=3. Grey lines indicate equilibrium soil respired $\delta^{13}\text{CO}_2 \pm 1$ standard error.

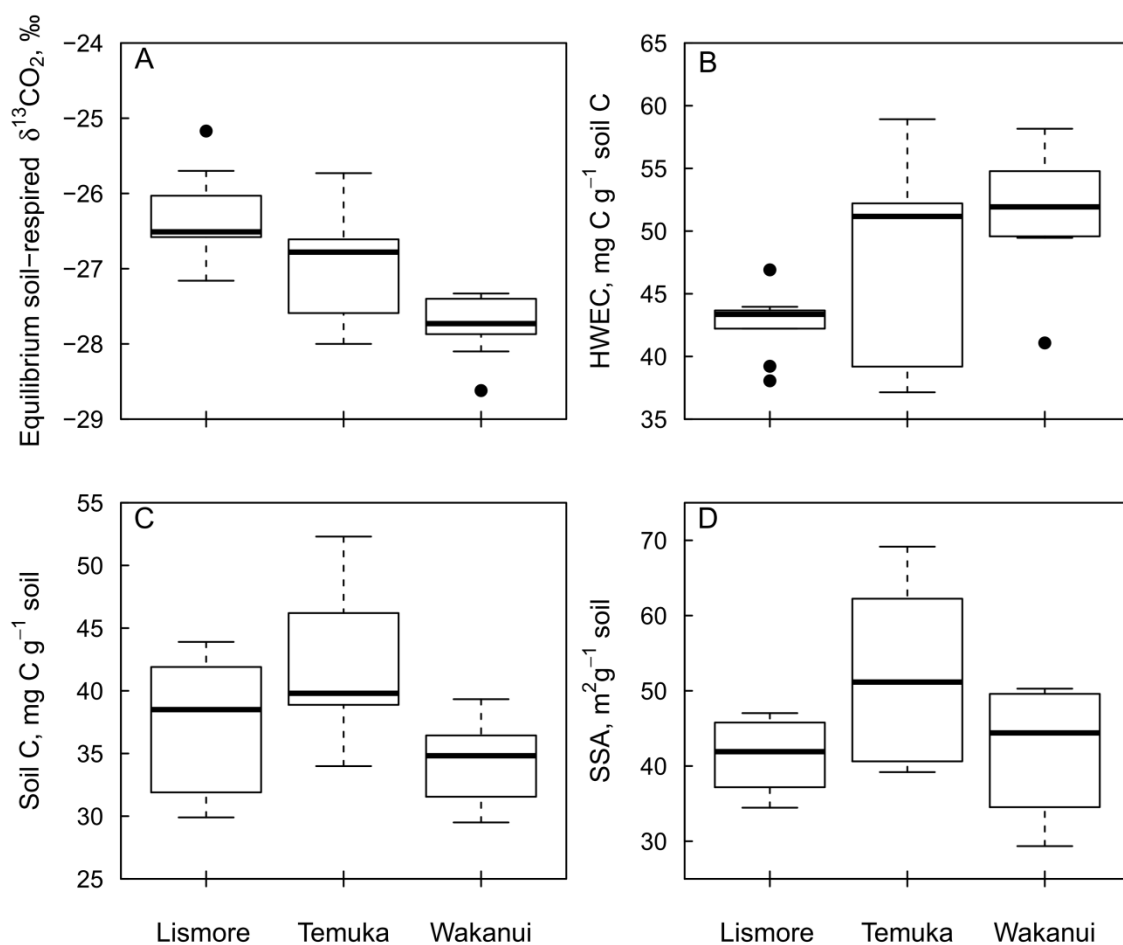


Figure 3.2. Box-and-whisker plots for equilibrium soil-respired $\delta^{13}\text{CO}_2$ (A), hot water extractable C (B), total soil C (C) and soil surface area (D) across the three soils: Lismore, Temuka and Wakanui. The plot whiskers extend to the most extreme data point which is no more than 1.5 times interquartile range from the box and the dots show outliers beyond this range.

Table 3.2. Soil carbon properties

Site	Equilibrium soil-respired $\delta^{13}\text{CO}_2$, ‰	Total soil C, mg C g ⁻¹ soil	HWEC ^a , mg C g ⁻¹ soil C	SSA ^a , m ² g ⁻¹ soil	POM C ^a , mg C g ⁻¹ soil C	Coarse silt C, mg C g ⁻¹ soil C	Fine silt C, mg C g ⁻¹ soil C	Clay C, mg C g ⁻¹ soil C
1 ^b	-25.7 ^c (0.34)	31 (0.7)	41 (1.8)	36.2 (0.85)	123 (10.5)	51 (0.2)	148 (1.3)	591 (12.7)
2	-26.5 (0.28)	42 (1.0)	43 (0.2)	46.4 (0.36)	140 (10.5)	62 (6.3)	184 (2.4)	523 (6.5)
3	-26.7 (0.21)	39 (1.9)	43 (2.3)	42.0 (0.36)	118 (17.2)	44 (5.5)	190 (10.2)	607 (13.9)
4 ^b	-26.6 (0.11)	40 (0.2)	38 (0.6)	40.0 (0.44)	105 (6.6)	57 (4.0)	193 (2.8)	556 (6.0)
5	-26.6 (0.52)	49 (1.9)	54 (2.5)	65.9 (2.01)	111 (2.2)	46 (7.0)	175 (2.1)	581 (13.4)
6	-27.8 (0.12)	36 (1.5)	53 (2.4)	50.2 (0.97)	115 (11.4)	32 (4.5)	163 (4.5)	612 (11.5)
7 ^b	-28.0 (0.35)	31 (1.0)	54 (1.3)	31.1 (1.71)	130 (2.2)	48 (1.2)	157 (9.5)	564 (5.5)
8	-27.7 (0.04)	37 (2.5)	54 (2.5)	50.0 (0.21)	115 (11.4)	41 (1.2)	168 (5.7)	558 (28.8)
9	-27.4 (0.13)	36 (0.5)	48 (3.3)	44.8 (0.42)	91 (1.8)	35 (2.1)	163 (1.6)	632 (4.8)

^aHWEC – hot water extractable carbon; SSA – soil surface area; POM C – carbon in the particulate organic matter

^bSoils from sites 1, 4 and 7 were used for the allophane addition experiment.

^cMean values (\pm 1 standard error, n=3).

The other models received considerably less support ($\Delta AICc_i > 5.3$). The best single-variate model included HWEC as a fixed effect ($\Delta AICc_i = 5.9$, **Table 3.3**, **Figure 3.3A**, $R^2 = 0.76$).

Table 3.3. Summary of the models fitted to soil properties to explain the equilibrium soil-respired $\delta^{13}\text{CO}_2$

Rank	Formula	d.f. ^a	AICc _i ^b	$\Delta AICc_i$ ^c	w _i ^d	cum. w. ^e	LogLik ^f
1	$y_0 \sim \text{HWEC} * \text{SoilC} * \text{SSA}$	11	54.05	0	0.85	0.85	-7.23
2	$y_0 \sim \text{HWEC} * \text{SoilC}$	7	59.44	5.39	0.06	0.91	-19.77
3	$y_0 \sim \text{HWEC}$	5	59.94	5.89	0.04	0.96	-23.54
4	$y_0 \sim \text{SoilC}$	5	62.52	8.47	0.01	0.97	-24.83
5	$y_0 \sim \text{HWEC} + \text{POMC}$	6	63.08	9.03	0.01	0.98	-23.44
6	$y_0 \sim \text{SSA}$	5	64.48	10.43	0	0.98	-25.81
7	$y_0 \sim \text{POMC}$	5	64.50	10.45	0	0.99	-25.82
8	$y_0 \sim \text{ClayC}$	5	64.55	10.50	0	0.99	-25.85
9	$y_0 \sim \text{Clay}$	5	64.79	10.74	0	1	-25.97
10	$y_0 \sim \text{HWEC} * \text{SoilC} * \text{Clay}$	11	66.17	12.12	0	1	-13.29
11	$y_0 \sim \text{HWEC} + \text{POMC} * \text{SoilC}$	8	67.34	13.29	0	1	-21.67
12	$y_0 \sim \text{POMC} * \text{SoilC}$	7	68.96	14.91	0	1	-24.54
13	$y_0 \sim \text{HWEC} * \text{POMC} * \text{SoilC}$	11	69.82	15.77	0	1	-15.11
14	$y_0 \sim \text{HWEC} * \text{POMC} * \text{SSA}$	11	74.48	20.43	0	1	-17.44
15	$y_0 \sim \text{POMC} * \text{SoilC} * \text{Clay}$	11	79.22	25.17	0	1	-19.81
16	$y_0 \sim \text{POMC} * \text{SoilC} * \text{SSA}$	11	80.90	26.85	0	1	-20.65
17	$y_0 \sim \text{HWEC} * \text{POMC} * \text{ClayC}$	11	82.41	28.35	0	1	-21.40

^ad.f. – degrees of freedom

^bAICc_i – Akaike's information criterion corrected for small sample sizes

^c $\Delta AICc_i = AICc_i - AICc_{\min}$, where $AICc_{\min}$ is the model with the smallest AICc

^dw_i – Akaike weight

^ecum.w. – sum of w_i

^fLogLik – log-likelihood of the fitted-model

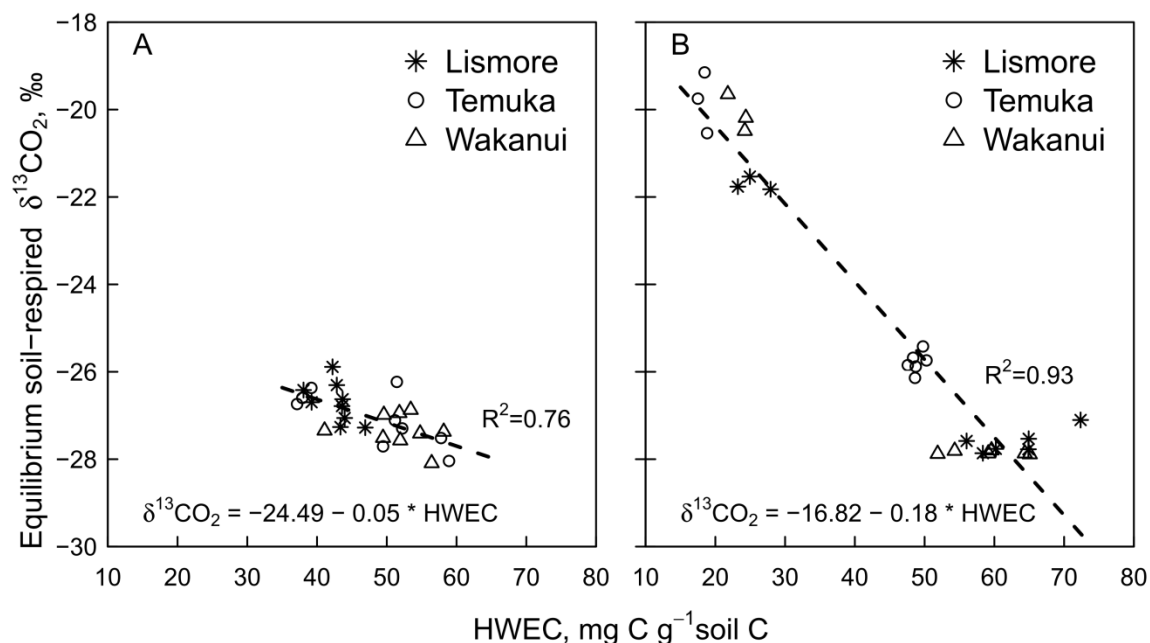


Figure 3.3. (A) Equilibrium soil-respired $\delta^{13}\text{CO}_2$ for the $y_0 \sim \text{HWEC}$ model for the pastures across the three soils; and (B) Relationship between equilibrium soil-respired $\delta^{13}\text{CO}_2$ and HWEC in the allophane addition experiments for the three selected paddocks. Equilibrium soil-respired $\delta^{13}\text{CO}_2$ in both graphs are given by the response minus the random effects from the fitted model.

3.3.3. Allophane addition experiment

Following allophane addition, soil-respired CO_2 became more enriched in ^{13}C during the first six days of incubation and stabilised at isotopic values which were significantly more enriched than the control treatments, across all soils (**Figure 3.4** and **Table 3.4**). Isotopic signatures of the soil-respired CO_2 in the two control treatments – soil alone and soil with sand addition – remained stable and relatively depleted in ^{13}C (**Figure 3.4** and **Table 3.4**) during the entire incubation. HWEC after allophane addition was up to three times less than from the control treatments (**Table 3.4**). HWEC extracted from allophane and sand was negligible ($< 5 \mu\text{g C/g}$ substrate). A linear correlation ($R^2 = 0.93$) was observed between the amount of HWEC and equilibrium (after stabilisation) soil-respired $\delta^{13}\text{CO}_2$ (**Figure 3.3B**).

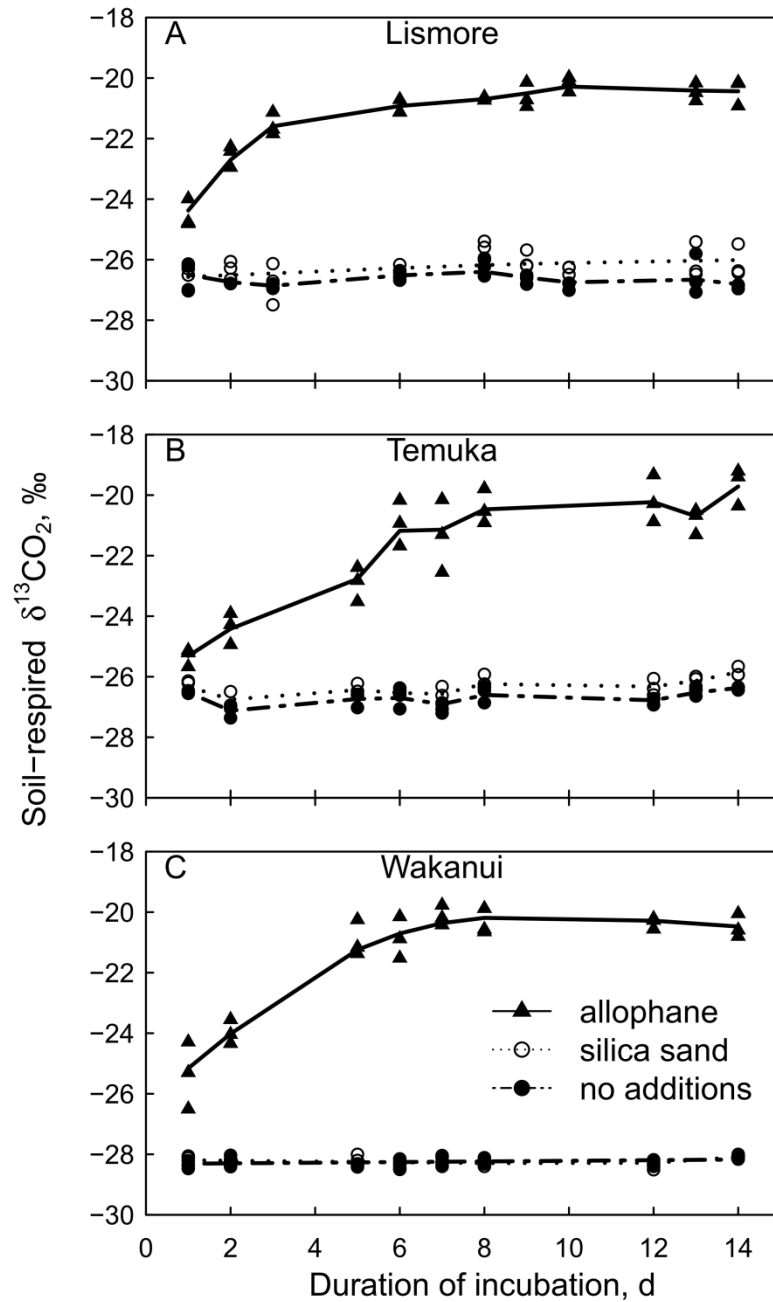


Figure 3.4. Isotopic composition of soil-respired $\delta^{13}\text{CO}_2$ incubated without any additions (●) and after addition of allophane (▲) or silica sand (○) from the three selected paddocks on (A) Lismore, $n=3$, (B) Temuka, $n=3$, and (C) Wakanui soil, $n=3$. The lines for each treatment were plotted for visualisation purposes only (using the smooth.spline function). Data for the Figure 3.4C were originally used in Chapter 2 of this thesis and are re-used here for comparison with the other soils.

Table 3.4. Equilibrium (after stabilisation) soil-respired $\delta^{13}\text{CO}_2$ in allophane addition, sand addition and no additions treatments, and hot water extractable carbon (HWEC) after 14 days of incubation for Lismore, Temuka and Wakanui pasture soils

Treatments	Lismore paddock		Temuka paddock		Wakanui paddock ^a	
	Equilibrium soil respired $\delta^{13}\text{CO}_2$, ‰	HWEC, mg C g ⁻¹ soil C	Equilibrium soil respired $\delta^{13}\text{CO}_2$, ‰	HWEC, mg C g ⁻¹ soil C	Equilibrium soil respired $\delta^{13}\text{CO}_2$, ‰	HWEC, mg C g ⁻¹ soil C
Allophane addition	-20.6 ^b (0.09)	25 (1.4)	-20.6 (0.40)	18 (0.4)	-20.5 (0.25)	23 (0.8)
Sand addition	-26.3 (0.15)	64 (4.7)	-26.4 (0.10)	49 (0.6)	-28.3 (0.03)	58 (3.6)
No additions	-26.7 (0.03)	61 (1.9)	-26.7 (0.09)	48 (0.4)	-28.3 (0.02)	60 (3.1)

^aResults for the Wakanui paddock were originally presented in Chapter 2 of this thesis and are re-presented here for comparison with the other soils.

^bMean values (\pm standard error, n=3).

3.4. Discussion

The trend in soil-respired $\delta^{13}\text{CO}_2$, observed within hours of soil disturbance, was most likely caused by changes in microbial substrate utilisation with relatively depleted, labile SOM becoming available. Labile SOM, which was previously inaccessible and protected by soil physical structures, became vulnerable to loss as a result of the soil disturbance. Models were fitted to the relationships between equilibrium soil-respired $\delta^{13}\text{CO}_2$ and soil properties and observed that the soil-respired $\delta^{13}\text{CO}_2$ values were a function of SOM amount and availability in soils. This interpretation was confirmed with an experimental approach which immobilised labile SOM onto active surfaces of allophane. These results were consistent with shifts in $\delta^{13}\text{CO}_2$ indicating both the amount of labile SOM and its availability in soils. Below, I discuss how these results contribute to our understanding of soil properties controlling labile SOM availability and potential vulnerability to loss.

3.4.1. Short-term soil incubations

A rapid change in $\delta^{13}\text{CO}_2$ was observed for all soils immediately following sampling (**Figure 3.1**). This trend was similar to the one described by Millard *et al.* (2010) and Zakharova *et al.* (2014) and was caused by changes in the physical protection of labile SOM due to disturbance of soil aggregates (especially macro-aggregates) and oxygen diffusion into the soil. Regular disturbance often leads to SOM depletion (Six *et al.*, 2002; Krull *et al.*, 2003), which is ascribed to a loss of C-rich macro-aggregates and an increase of C-depleted micro-aggregates in soils with an aggregate hierarchy (Six *et al.*, 2000). Thus, labile SOM, which is known to be mainly associated with macro- rather than with micro-aggregates in temperate grassland soils (Elliott, 1986), can be quickly lost after soil disturbance. In our study, this release caused the rapid change and then stabilisation of soil-respired $\delta^{13}\text{CO}_2$. The trend in soil-respired $\delta^{13}\text{CO}_2$ was not due to isotopic disequilibrium effects (Gamnitzer *et al.*, 2011), as discussed previously (Zakharova *et al.*, 2014).

3.4.2. Relationships between $\delta^{13}\text{CO}_2$ and soil properties

To test the hypothesis, that during short-term soil incubations, equilibrium soil-respired $\delta^{13}\text{CO}_2$ is a direct function of the amount of labile SOM and soil physical conditions, models were fitted to the relationships between equilibrium soil-respired $\delta^{13}\text{CO}_2$ and various soil properties. Some properties (such as HWE, POM) are used as traditional measures of labile SOM, whereas others (clay content and SSA) – are likely to determine its availability and soil protection capacity against SOM loss.

Total soil C content partially governs the amount of labile SOM available in soils, as unprotected SOM is usually accumulated only when soil C content is higher than soil protection level. This soil protection level is considered to be determined by silt and clay particles, micro-aggregates and condensation/ complexation reactions (Hassink *et al.*, 1997; Six *et al.*, 2002). So, at a low total soil C content, a large proportion of C is likely to be protected. HWE (a widely-used measure of labile SOM based on solubility) gave a good correlation with equilibrium soil-respired $\delta^{13}\text{CO}_2$. However, POM, which is also considered as a physically separated, labile fraction, did not appear to be as important. POM was reported to have a higher potential for loss after a disturbance (Cambardella & Elliott, 1992); however, silt- and clay-associated C can also be lost (Six *et al.*, 2002). This observation might explain why POM was not the soil property that explained the majority of the variability in equilibrium soil-respired $\delta^{13}\text{CO}_2$.

The models that included the proportion of clay particles in the soils did not receive much support from the data (**Table 3.3**). Silt and especially clay particles provide active mineral surfaces capable of binding labile substrates and protecting them against potential loss due to physicochemical stabilisation mechanism (Six *et al.*, 2002; Krull *et al.*, 2003). However, silt and clay content did not account for all variability in soil C content across a wide range of soils (Six *et al.*, 2002; Feng *et al.*, 2013). Indeed, Percival *et al.* (2000)

observed a very poor correlation ($R^2 < 0.05$) between clay content and soil C in New Zealand grassland soils. Instead, they reported a much better correlation between soil C content and pyrophosphate-extractable aluminium (Al) ($R^2 = 0.55$) and concluded that chemical stabilisation of SOM was the key process controlling soil C accumulation. C content of the fine ($< 20 \mu\text{m}$) fraction was not an important variable in our analyses. Protection of SOM by aggregates and bonding strength of C to clay surfaces (physico-chemical stabilisation) have been reported to complement each other (Krull *et al.*, 2003). Franzluebbers and Arshad (1996) observed a strong positive correlation between macro-aggregation and clay content in conventionally tilled soils and, in a subsequent study, reported an increase in physical protection of POM within aggregates as clay content increased (Franzluebbers & Arshad, 1997). Feng *et al.* (2013) proposed an alternative approach for SOM stabilisation based on C stabilisation on soil mineral surfaces. Nevertheless, it is worth mentioning that SSA is a function of both particle size distribution and mineralogical composition, and is a co-related property to SOM content as polar water molecules tend to associate with SOM surfaces. However, SSA may still be a useful indicator of SOM protection capacity and changes in SOM bioavailability.

Based on the above, SSA was hypothesised to help explain the variability in the equilibrium soil-respired $\delta^{13}\text{C}\text{O}_2$. SSA, as a fixed effect on its own, did not provide strong explanatory power. However, a model that included the interaction between HWEC, total soil C and SSA provided the best fit to the equilibrium soil-respired $\delta^{13}\text{C}\text{O}_2$ (**Table 3.3**). This model took into account not only the amount of labile SOM (measured as HWEC and influenced by total soil C) but also soil potential for C protection (indicated by SSA). Labile SOM has a relatively depleted ^{13}C isotopic signature, (close to initial plant material) compared with bulk soil (Balesdent *et al.*, 1987; John *et al.*, 2005; Gunina & Kuzyakov, 2014). This is due to the fact that labile SOM has not yet undergone major biochemical

changes, during which fractionation occurs due to microbial metabolism discriminating against the heavier ^{13}C (Ehleringer *et al.*, 2000; Werth & Kuzyakov, 2010). Hence, the more labile SOM is available in the soil (with HWEF being the estimate of the labile fraction), the more depleted is soil-respired $\delta^{13}\text{CO}_2$. Clay particles largely determine SSA by providing active mineral surfaces capable of binding labile substrates (Six *et al.*, 2002; Krull *et al.*, 2003). Furthermore, more recalcitrant, mineral fraction (mainly clay-associated C) is the most microbially processed and thus relatively enriched in ^{13}C (Gunina & Kuzyakov, 2014). Thus, an increase in SSA leads to a decrease in labile SOM with an increase in the more recalcitrant SOM fraction, ultimately resulting in a relative enrichment in ^{13}C of the equilibrium soil-respired $\delta^{13}\text{CO}_2$ values. So, the shifts in soil-respired $\delta^{13}\text{CO}_2$ are a direct function of the amount of labile SOM and soil physical conditions, which in turn are governed by soil properties. In particular, equilibrium soil-respired $\delta^{13}\text{CO}_2$ indicates not only the amount of labile SOM, but also its availability in soils, mainly determined by soil surface area. I also suggest that the equilibrium soil-respired $\delta^{13}\text{CO}_2$ might be a measure of labile SOM vulnerability to loss after soil disturbance. However, in this study I did not test this hypothesis directly as I did not quantify the amount of labile SOM being respired. So I did not directly correlate the two soil respiration-based measures – equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the amount of labile SOM being respired.

3.4.3. Allophane addition experiments

To test the effect of specific soil surface area on the equilibrium soil-respired $\delta^{13}\text{CO}_2$, additional soil was incubated with allophane, which is capable of binding organic compounds through a specific ligand exchange mechanism between carboxyl and carbohydrate groups of SOM and the hydroxyl groups in allophane (Parfitt *et al.*, 1999). As labile SOM was bound to allophane, soil-respired $\delta^{13}\text{CO}_2$ became relatively more enriched in ^{13}C compared to the control treatments. Reduced availability of labile SOM after allophane addition was confirmed with a significant reduction in HWEC. I suggest that the difference in $\delta^{13}\text{CO}_2$ between allophane amendment and control treatments demonstrates the effect of specific soil surface area in reducing microbial accessibility and protecting labile SOM in soils. Overall, the allophane addition experiments proved earlier observations of the relationships between equilibrium soil-respired $\delta^{13}\text{CO}_2$ and soil properties, clearly indicating that an increase in SSA leads to a relative enrichment in ^{13}C of the equilibrium soil-respired $\delta^{13}\text{CO}_2$ through a decrease in labile SOM.

3.5. Summary

The isotopic analysis of soil-respired CO₂ provides us with a tool to assess labile SOM dynamics and availability after soil disturbance, which is often caused by changes in land use. The results presented in this chapter showed that shifts in soil-respired $\delta^{13}\text{CO}_2$ during incubation indicated the amount of labile SOM and soil physical conditions. In particular, equilibrium soil-respired $\delta^{13}\text{CO}_2$ was a function of both labile SOM amount and its availability in soils. The amount of labile SOM in soils was based on the HWEC estimate, while labile SOM availability was determined by soil surface area. Higher soil surface area resulted in a decrease in HWEC (as an estimate of the labile SOM) and a relative enrichment in ^{13}C of the equilibrium soil-respired $\delta^{13}\text{CO}_2$, as demonstrated by incubating soil with allophane. Thus, I have been able to link isotopic composition of the soil-respired CO₂ with the traditional measures of SOM amount and soil physical conditions. I also think that the equilibrium soil-respired $\delta^{13}\text{CO}_2$ might be a quick measure of labile SOM vulnerability to loss; however, this hypothesis needs to be directly tested by quantifying the amount of respired labile SOM during soil incubations.

CHAPTER 4: LABILE SOIL ORGANIC MATTER
AVAILABILITY ASSESSED THROUGH FRACTIONATION
METHODS AND SOIL-RESPIRED $\delta^{13}\text{CO}_2$

4.1. Introduction

Labile soil organic matter (SOM) consists of readily decomposable compounds with fast turnover rates (Townsend *et al.*, 1997; Krull *et al.*, 2003) and is the main source of nutrients released following soil disturbance (Elliott, 1986; Cambardella & Elliott, 1992). Undisturbed soils with a regular input of plant carbon (C) accumulate labile SOM that may be attributed to a build-up of particulate organic matter (POM), an increase in microbial biomass and/or the protection of the SOM by soil aggregates (Beare *et al.*, 1994; Golchin *et al.*, 1994; Six *et al.*, 2002; Krull *et al.*, 2003; von Lützow *et al.*, 2006). Mechanical soil disturbance, such as regular agricultural tillage, breaks up aggregate structures and increases SOM availability to oxidation and microbial attack. As a result labile, but no longer physically protected, SOM can be quickly lost, leading to a significant depletion in total SOM in cultivated soils as reviewed by Balesdent *et al.* (2000). However, Curtin *et al.* (2014), who carried out a study on a tillage trial site with long-term management treatments, which was also used in this research, concluded that the decreased amount of plant C inputs after converting pasture to arable cropping was the main factor responsible for the decline in SOM content. Due to regular plant C inputs and enhanced SOM accumulation in the absence of soil disturbance, grassland soils usually have more SOM and are closer to their ultimate C saturation level compared with the cultivated soils (Stewart *et al.*, 2008a). This soil C saturation level is controlled by physicochemical characteristics of various SOM pools, which are (i) unprotected, (ii) physically protected by aggregates, (iii) chemically associated with clay and silt particles, and (iv) biochemically protected by organic compounds (Six *et al.*, 2002; Stewart *et al.*, 2008a; Stewart *et al.*, 2008b). Overall, physical protection and microbial accessibility of labile SOM are more important for determining the rate of SOM turnover than intrinsic recalcitrance (Dungait *et al.*, 2012).

A number of methods have been used to measure the amount of labile SOM in soils. Procedures rely on chemical, physical or biological fractionation or a combination to separate the labile SOM before measurement (McLauchlan & Hobbie, 2004; von Lützow *et al.*, 2007). Water soluble carbon (WSC) and hot water extractable carbon (HWE) are common measures of labile SOM, based on chemical solubility (Ghani *et al.*, 2003; Haynes, 2005). Physical fractionation methods include density and particle size fractionation and can assess spatial arrangements of organo-mineral complexes, as particles of different sizes have different structures and functions (Christensen, 2001). Sand-sized SOM, also known as particulate organic matter (POM), or the light fraction of SOM obtained by density fractionation, are both rapidly mineralised and quickly lost as a result of changes in management and thus are widely regarded as labile SOM fractions (Cambardella & Elliott, 1992; Balesdent, 1996; Christensen, 2001). Silt and clay particles contribute to SOM protection as they provide reactive surfaces for physicochemical stabilisation and determine soil surface area (SSA) (Six *et al.*, 2002; Krull *et al.*, 2003). Biological fractionation assumes that microbes mineralize labile SOM first, and separates the pools by allowing microbes to mineralize SOM without fresh organic inputs (Townsend *et al.*, 1997; Alvarez & Alvarez, 2000; McLauchlan & Hobbie, 2004).

An alternative to fractionation methods for measuring labile SOM is the use of C isotopes and ^{13}C signatures in particular. Stable C isotopes have been widely used in ecosystem studies to quantify C flows and SOM dynamics and partition soil respiration either after a transition between C_3 and C_4 vegetation (Balesdent *et al.*, 1987; Balesdent *et al.*, 1988; Cadisch *et al.*, 1996; Townsend *et al.*, 1997; Millard *et al.*, 2008; Blagodatskaya *et al.*, 2011) or by applying a label with a different ^{13}C signature (Pendall & King, 2007; Dorodnikov *et al.*, 2011). An approach based on ^{13}C natural abundance relies on the differences in isotopic composition likely due to preferential microbial decomposition and microbial fractionation

(Ehleringer *et al.*, 2000; Werth & Kuzyakov, 2010). Millard *et al.* (2010) used ^{13}C signatures of soil-respired CO_2 as a tool for measuring labile SOM and found a rapid depletion of respired $\delta^{13}\text{CO}_2$ within a few hours of incubation. Zakharova *et al.* (2014) confirmed that shifts in soil-respired $\delta^{13}\text{CO}_2$ over the course of soil incubations reflected changes in the use of labile substrate. A rapid initial depletion of $\delta^{13}\text{CO}_2$ suggested an increased availability of labile SOM, and a subsequent reversion back to the relatively enriched values indicated that labile SOM pools were exhausted (Zakharova *et al.*, 2014). Thus, short-term changes in soil-respired $\delta^{13}\text{CO}_2$ are likely to be a direct function of the amount of labile SOM and the level of its protection. Using natural differences in the ^{13}C isotopic composition of aggregates and SOM fractions, Gunina and Kuzyakov (2014) studied flows between SOM fractions and aggregates and confirmed successive steps of SOM formation. They observed continuous ^{13}C enrichment between the SOM fractions, with the most labile fraction being close to initial plant material and depleted in ^{13}C and the mineral fraction being most processed and enriched. They standardised the isotopic composition of the density fractions against the solid soil material and calculated $\Delta^{13}\text{C}$ values, as the difference between $\delta^{13}\text{C}$ values of density fractions and the solid soil. It allowed them to confirm the direction of C flows from the free POM to the mineral fractions (Gunina & Kuzyakov, 2014).

In this study, $\Delta^{13}\text{CO}_2$ calculations were combined with soil incubations to test the hypothesis that the isotopic composition of soil-respired CO_2 is a measure of labile SOM vulnerability to loss, following soil disturbance. Soils from different long-term management treatments including permanent pasture, chemical fallow and arable cropping were sampled from a tillage experiment, which had also been used for a study in 2005 by Curtin *et al.* (2014). Soils were incubated in the short- (300 minutes) and in long-term (600 days), and changes in soil-respired $\delta^{13}\text{CO}_2$ and respiration rates were measured. In addition, various soil properties (such as total soil C, WSC, HWE, SSA, sand, silt and clay content, $\delta^{13}\text{C}$ and C

content of soil fractions) were quantified and $\Delta^{13}\text{CO}_2$ values calculated. Models were fitted to the relationships between the standardised against solid soil material isotopic composition of soil-respired CO_2 ($\Delta^{13}\text{CO}_2$) and various soil properties, including traditional estimates of labile SOM, to test the main hypothesis of the study that the proportion of labile C respired over the course of the incubations provided the best predictive value to $\Delta^{13}\text{CO}_2$. Additionally, the effect of long-term regular C inputs and mechanical soil disturbance regimes on labile SOM pools and their vulnerability to loss was also investigated.

4.2. Materials and methods

4.2.1. Study site

Soil was sampled (2011) at the Millennium Tillage Trial (MTT), a field site established by the New Zealand Institute for Plant and Food Research in November 2000 near Lincoln, New Zealand (43° 40' S, 172° 28' E, 5 m above sea level). Soil at the site was classified as a Wakanui silt loam (Hewitt, 2010) (USDA classification, Aquic Haplustept). The site had previously been under irrigated sheep-grazed permanent pasture and had not been cultivated for at least 14 years prior to setting up the trial (Fraser *et al.*, 2010). The treatments sampled in early winter (May – June 2011) were:

- chemical ‘fallow’: no soil cultivation or fertilisation, bare soil surface was maintained (by spraying with glyphosate) during the entire trial;
- ‘intensive tillage’ with autumn sown barley (*Hordeum vulgare* L.) cultivar Retriever: autumn soil cultivation to 200 mm using a mouldboard plough, followed by secondary cultivation (two passes with a spring-tined implement, each followed by harrowing and rolling); crop direct drilled, no additional winter cover crop;

- ‘no-till’ with autumn sown barley (*Hordeum vulgare* L.) cultivar Retriever: no soil cultivation, crop direct drilled, no additional winter cover crop;
- ‘permanent pasture’ (with ryegrass, *Lolium perenne* L. and clover, *Trifolium repens* L.), grazed by sheep (pre-trial land use; maintained as a control treatment in the trial).

Each treatment had three replicated plots, and three replicated soil cores were taken from each plot.

4.2.2. Soil sampling and incubations

The protocol used for soil sampling and soil incubations was the same as in Zakharova *et al.* (2014). Soil cores (50 mm diameter) were taken to a depth of 250 mm, brought intact to the laboratory, broken open and visible roots and any stones quickly (within minutes) removed by hand and discarded. The remaining soil from each core was mixed and placed in a Tedlar® bag (Keika Ventures, Chapel Hill, NC, USA). The bag was sealed, and then quickly and repeatedly flushed with CO₂-free air (typically 5-6 times) until less than 20 ppm CO₂ remained in the bag. Samples were incubated *in situ* at an ambient temperature (18 – 22°C) and aliquots of gas were regularly removed to check the CO₂ concentration with a portable infra-red gas analyser (IRGA EGM-4, PP Systems, Hitchin, UK). When at least 350 ppm CO₂ had accumulated (typically after 7 – 10 minutes), the headspace gas was transferred to a second Tedlar bag and then analysed for $\delta^{13}\text{CO}_2$, as described below. The bag with the soil was then flushed with CO₂-free air again and re-incubated until sufficient CO₂ had again accumulated for a second sample. Sampling was repeated periodically during 300 minutes of incubations, allowing the isotopic signatures of the respired CO₂ to stabilise. After soil incubations were finished, 250 g of soil (from a randomly selected core per plot) was collected for chemical and physical analyses.

The remaining soil samples were transferred into plastic pots without lids, and were kept in an incubator at a constant temperature (30°C) and water content (field moisture, watered twice a week to constant weight) for 600 days ('long-term incubation'). During this time, the evolution rates of respired CO₂ were measured first weekly, and then monthly by connecting the pots to the IRGA EGM-4.

To assess the influence of depth on the soil-respired $\delta^{13}\text{CO}_2$ we collected additional soil cores (50 mm diameter) to a depth of 250 mm (one core from each replicate plot) for the four treatments. The cores were brought intact to the laboratory, where they were cut into 'topsoil' 0 – 150 mm and 'subsoil' 150 – 250 mm layers. Each of the cores was individually incubated in the short-term in Tedlar bags and gas samples collected for $\delta^{13}\text{CO}_2$ analysis.

4.2.3. Isotopic measurements

Bags with gas samples were connected to a tunable diode laser, TDL (TGA100A; Campbell Scientific Inc., Logan UT, USA), which measures concentrations of the three CO₂ isotopologues ($^{12}\text{C}^{16}\text{O}_2$, $^{13}\text{C}^{16}\text{O}_2$ and $^{12}\text{C}^{16}\text{O}^{18}\text{O}$) at 1 Hz (Bowling *et al.*, 2003). The $^{13}\text{C}/^{12}\text{C}$ ratios (R) were converted to delta notation ($\delta^{13}\text{C}$, in ‰) relative to the Vienna Pee Dee Belemnite standard (V-PDB) as described by Barbour *et al.* (2007) using Equation (2.1). Typical precision for isotope analysis was ± 0.02 ‰ (± 1 standard error (SE), $n = 45$). The $\delta^{13}\text{C}$ values of the solid soil material and soil fractions were determined using a continuous flow isotope ratio mass spectrometer (Thermo Finnigan Delta Plus^{advantage}) interfaced to an elemental analyser (Thermo FlashEA1112, Thermo Fisher, Bremen, Germany).

4.2.4. Physical fractionation

The particle size analysis was adapted from Gee and Bauder (1986). Air-dried soil (20 g; < 2 mm) was dispersed in 60 ml of deionised (DI) water using an ultrasonic probe (Bandelin SONOPULS HD 2200, mean power output 63.6 W for 60 s). The suspension was washed through a 53 μm sieve (retaining the sand fraction on the sieve). The <53 μm material

was further separated into 53 – 5 μm (silt) and < 5 μm (clay) fractions by gravity sedimentation, based on particle settling times calculated by Stoke's law (Gee & Bauder, 1986). Solid $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ (2 g per litre of suspension) was used for clay flocculation. All soil fractions were dried in the oven (60 °C), weighed and ground for total C analysis on a LECO TruMac CN analyser (USA) based on the Dumas method (Leco Corporation, 2003) with a combustion temperature of 1250 °C.

4.2.5. Water extractions

The protocol used for water extractions was adapted from the method of Ghani et al. (2003). Tubes with air-dried soil (3 g; < 2 mm) and 30 ml of DI water were shaken and centrifuged (30 minutes and 20 minutes at 3500 rpm, respectively). The supernatant was filtered through a pre-leached Advantec 5C filter paper into vials for water soluble carbon (WSC) analysis. The tubes were weighed and another 30 ml of DI water was added to the same tubes. The tubes were shaken thoroughly to re-suspend the soil samples, placed in a hot water bath at 80 °C for 16 hours, and then centrifuged for 20 minutes at 3500 rpm. The supernatant was filtered for hot water extractable carbon (HWEC) analysis. WSC and HWEC were measured on a Shimadzu TOC - 5000A analyser (Tokyo, Japan). Additional hot water extractions (180 g soil, 1800 ml DI water) were prepared, freeze-dried in a VirTis Genesis 25L freeze-drier (NY, USA) and the dry powder was analysed for $\delta^{13}\text{C}$ of HWEC, as described above.

4.2.6. SSA measurements

The specific soil surface area (SSA, $\text{m}^2 \text{g}^{-1}$) was calculated from the water content of air-dried soil samples using the linear regression reported by Parfitt *et al.* (2001), using Equation (3.1), where W_A was the water content of air-dried soil samples (g kg^{-1}), estimated by the weight difference between the air-dried sample (30 °C for 24 hours) and the subsequently oven-dried sample (105 °C for 24 hours to a constant weight).

4.2.7. Statistical analyses

An exponential decay function was fitted to the time sequence $\delta^{13}\text{CO}_2$ data from the short-term incubation for each soil core:

$$\delta^{13}\text{CO}_2 = y_0 + a_1 * \exp^{-b_1 * t} \quad (4.1)$$

where y_0 represents the equilibrium $\delta^{13}\text{CO}_2$ value in the short-term incubation duration t in minutes, and a_1 and b_1 characterise the shape of the exponential decay function.

The approach described by Gunina and Kuzyakov (2014) was followed to standardise the isotopic composition of the equilibrium soil-respired $\delta^{13}\text{CO}_2$ against the solid soil material. The standardised isotopic composition was calculated as the difference ($\Delta^{13}\text{CO}_2$, in ‰) between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ values (y_0) and $\delta^{13}\text{C}$ of the solid soil material:

$$\Delta^{13}\text{CO}_2 = y_0 - \delta^{13}\text{C soil}. \quad (4.2)$$

Evolution rates (E) of respired CO_2 ($\text{g CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$) were expressed as daily C mineralisation rates ($\mu\text{g C g}^{-1} \text{ soil d}^{-1}$):

$$\text{C mineralisation rate} = (24 * 0.2727 * A * E * 10^6) / m \quad (4.3)$$

where A represents the surface area of the pot with soil (m^2), m is weight of dry soil (g), 24 is the recalculation coefficient for the daily rates, and 0.2727 is the conversion factor from CO_2 to C based on their relative atomic mass.

An exponential decay function was also fitted to the time sequence of the C mineralisation rate during the long-term incubation:

$$\text{C mineralisation rate} = C_0 + a_2 * \exp^{-b_2 * t} \quad (4.4)$$

where C_0 represents the steady mineralisation rate of C at time t in days, and a_2 and b_2 characterise the shape of the exponential decay function. Decay curves were fitted to each soil core using linear mixed-effects models (with plot as a random effect and parameters y_0 , C_0 , a_1 , a_2 , b_1 and b_2 were allowed to vary by treatment).

An estimate of the labile SOM pool – labile C respired – was determined following the approach described by Townsend *et al.* (1997). The total amount of mineralised C was determined by integrating the area under the exponential decay mineralisation curve between days 1 and 600. The amount of labile C was obtained by subtracting the amount attributable to more recalcitrant SOM respiration at a steady rate, after the stabilisation of the CO₂ flux. Finally, the proportion of labile C respired was obtained by dividing the amount of labile C by total soil C content. These calculations were based on the study by Townsend *et al.* (1997), where the authors ascribed the drop and stabilisation of soil respiration at a steady rate as a near complete loss of active, labile SOM.

Relationships between standardised $\Delta^{13}\text{CO}_2$ values and soil properties were analysed using linear mixed-effects models with treatment as a random intercept. A candidate set of models was established to identify which of the soil properties (such as WSC, HWEC, labile C respired, C content and $\delta^{13}\text{C}$ of particle-size fractions, SSA and total soil C content) explained the majority of the variability in the isotopic values. These models reflected plausible hypotheses about the relationships between $\Delta^{13}\text{CO}_2$, the amount of labile SOM and specific soil properties.

The candidate model set was built on the following hypotheses:

- The proportion of labile C respired explains $\Delta^{13}\text{CO}_2$, as both measures are based on SOM respiration and estimate respiratory losses.
- HWEC and sand-sized C (Sand C) give a good estimate of the amount of labile SOM as they are widely considered to be labile fractions (von Lützow *et al.*, 2007).
- Increased clay/silt content and SSA reduce the amount of labile SOM that is available and thus vulnerable to loss due to physicochemical stabilisation (Six *et al.*, 2002; Krull *et al.*, 2003).

- $\delta^{13}\text{C}$ values of the HWE C or Sand C fractions predict $\Delta^{13}\text{CO}_2$ as they give isotopic values of the labile SOM fractions which provide easily available substrate for soil respiration.

A similar candidate set of models was built on the hypotheses mentioned above to analyse the relationships between equilibrium soil-respired $\delta^{13}\text{CO}_2$ (y_0) and soil properties. Additionally, the relationship between y_0 and the proportion of labile C respired was analysed for the effect of treatment (fallow, intensive, no-till and pasture) using linear mixed-effects models (with replicate soil cores within plot as random intercept). The models, with and without treatment as a fixed effect, were compared using the Akaike's information criterion corrected for small sample sizes (AICc) to identify the best fit (see the section on AICc below).

Based on the results of this analysis, another candidate set of models was built to identify which of the traditional measures of labile SOM and other soil properties and their combinations best explained the variability in the proportion of labile C respired. By doing so I attempted to explain the large difference between the proportions of labile C respired from pastures compared to all other treatments. Consequently, this candidate model set was built on the following hypotheses:

- Total soil C increases the proportion of labile C respired as it partially governs the amount of labile SOM available (Six *et al.*, 2002).
- HWE C and Sand C are good estimates of the proportion of labile C respired as they measure labile SOM fractions providing the easily available substrate for soil respiration (von Lützow *et al.*, 2007).
- Clay/silt content, SSA and C content of the clay/silt fractions reduce the amount of labile SOM and thus the amount of labile C respired due to physicochemical stabilisation (Six *et al.*, 2002; Krull *et al.*, 2003).

- The combinations of HWEC/ Sand C, total soil C and SSA/ Clay C/ Silt C explain the proportion of labile C respired as they measure both the amount of labile SOM and its availability/vulnerability to loss (the results of the previous candidate model set).

All models were ranked using the Akaike's information criterion corrected for small sample sizes (AICc) to determine the Kullback-Leibler (KL) best model (Burnham & Anderson, 2002). The AICc identifies the model(s) most strongly supported by the data based on bias-corrected, maximised log-likelihood (LogLik) of the fitted-model with a penalty for the number of parameters used. The model with the smallest AICc ($AICc_{min}$) is the most strongly supported. $\Delta AICc$ is calculated for each model i as $\Delta i = AICc_i - AICc_{min}$. As a rule of thumb, models with Δi of < 2 also receive substantial support, models with $2 < \Delta i < 7$ receive considerably less support, whereas models with $\Delta i > 8-14$ receive no support (Anderson, 2008).

The post-hoc Tukey test ($\alpha = 0.05$) was used to test for significantly different values between treatments in all the experiments. The Welch Two Sample t-test ($\alpha = 0.05$) was used to test for significant differences between depth layers.

All analyses were performed in R (version 3.0.2, R Development Core Team, 2013) and included use of the packages *lme* and *nlme* (Pinheiro *et al.*, 2013).

4.3. Results

4.3.1. Initial short-term depletion of $\delta^{13}\text{CO}_2$

Soil-respired $\delta^{13}\text{CO}_2$ showed a similar pattern through time for all soil treatments. Initial values of $\delta^{13}\text{CO}_2$ (within a few minutes of the soil being sampled) were in the range -22.0 to -23.3‰ (**Figure 4.1**). During the incubation, $\delta^{13}\text{CO}_2$ became more depleted in ^{13}C and stabilised after 50 – 100 minutes at an equilibrium $\delta^{13}\text{CO}_2$ value (y_0), which was in the range -26.3 to -28.1‰, depending on the treatment (**Table 4.1**). The equilibrium soil-respired values (y_0) were significantly more enriched in ^{13}C for fallow soils than the other treatments (**Table 4.1**).

4.3.2. Depth influence on the equilibrium $\delta^{13}\text{CO}_2$

Depth did not alter this across-treatment trend: both layers of the fallow soils had significantly more enriched equilibrium soil-respired $\delta^{13}\text{CO}_2$ values (-24.7 ± 0.35 ‰ and -23.9 ± 0.64 ‰ for ‘topsoil’ and ‘subsoil’, respectively) compared with the values for the same depth layers of the pasture soils (-28.7 ± 0.09 ‰ and -27.3 ± 0.22 ‰, respectively); t-test results: $t = 11.0$, $df = 4$, $p = 0.0004$ and $t = 5.0$, $df = 4$, $p = 0.008$ respectively. The values obtained for the ‘topsoil’ and ‘subsoil’ layers under the intensive tillage treatment were not significantly different compared with the same depth layers of the no-till treatment (-27.3 ± 0.21 ‰ and -26.7 ± 0.44 ‰, for the ‘topsoil’ and ‘subsoil’ of the intensively ploughed soils, respectively, and -27.1 ± 0.24 ‰ and -26.1 ± 0.35 ‰, for the ‘topsoil’ and ‘subsoil’ of no-till soils, respectively); t-test results: $t = -0.7$, $df = 4.0$, $p = 0.5$ and $t = -1.1$, $df = 4$, $p = 0.3$ respectively. In addition, the ‘subsoil’ for each treatment was characterised by more enriched equilibrium $\delta^{13}\text{CO}_2$ values compared to the ‘topsoil’ layer. This trend of isotopic enrichment with depth was seen for all treatments, however, significant differences between the ‘topsoil’ and ‘subsoil’ layers were observed only for the pasture soils (t-test results: $t = -6.0$, $df = 4$, $p = 0.004$).

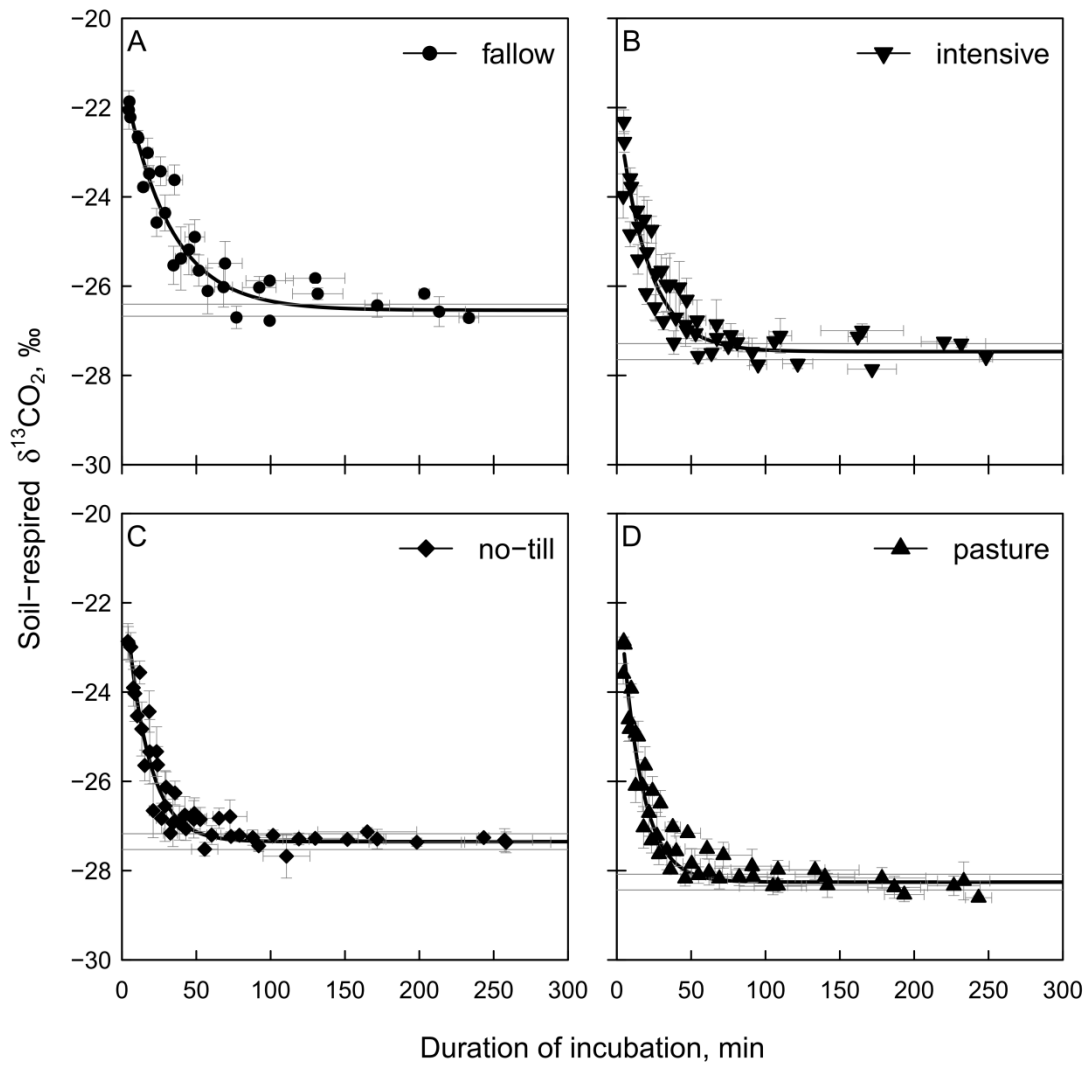


Figure 4.1. Isotopic composition of soil-respired CO_2 measured in short-term incubations with exponential decay model fitted for the (A) fallow, (B) intensive, (C) no-till and (D) pasture treatments ($n=3$). Data are presented as mean plot values ± 1 standard error. Grey lines indicate equilibrium soil-respired $\delta^{13}\text{CO}_2 \pm 1$ standard error. The best exponential decay model consisted of different equilibrium y_0 values and a and b coefficients for each treatment. Data in Figure 4.1C were first used in Chapter 2 and are re-used here for comparison with the other treatments.

Table 4.1. Isotopic composition of soil-respired CO₂ and solid soil material for the soils under fallow, intensive, no-till and pasture treatments.

Treatments	Equilibrium $\delta^{13}\text{CO}_2$ (y_0) ^a , ‰	$\delta^{13}\text{C}$ solid soil ^a , ‰	$\Delta^{13}\text{CO}_2$ ^a , ‰
Fallow	-26.3 ^b (0.11)a	-26.0 (0.08)a	-0.3 (0.08)a
Intensive	-27.5 (0.09)b	-26.7 (0.06)b	-0.8 (0.03)ab
No-till	-27.5 (0.10)b	-26.4 (0.08)ab	-1.1 (0.19)b
Pasture	-28.1 (0.38)b	-27.3 (0.12)c	-1.1 (0.14)b

^a y_0 – equilibrium soil-respired $\delta^{13}\text{CO}_2$; $\delta^{13}\text{C}$ of the solid soil; $\Delta^{13}\text{CO}_2$ – the isotopic difference between y_0 and $\delta^{13}\text{C}$ of the solid soil

^bMean values (\pm 1 standard error, $n = 3$, soil depth 0 – 250 mm). Different lower case letters indicate significant differences between treatments based on post-hoc Tukey test ($P < 0.05$).

4.3.3. C mineralisation rates

C mineralisation rates during the long-term soil incubations decreased exponentially as the incubation proceeded (**Figure 4.2**). The pasture soils had faster initial C mineralisation rates (2 to 5 times higher compared with both cropping soils and the fallow soil, respectively), which declined over the course of the incubation. It took 25 days for the mineralisation rates to level off to an approximate steady state for the fallow treatment, 50 days for intensive and no-till treatments and over 300 days for pasture soils. The steady-state mineralisation rate for pastures at the end of the incubation was 2 – 3.5 times higher compared with the other treatments.

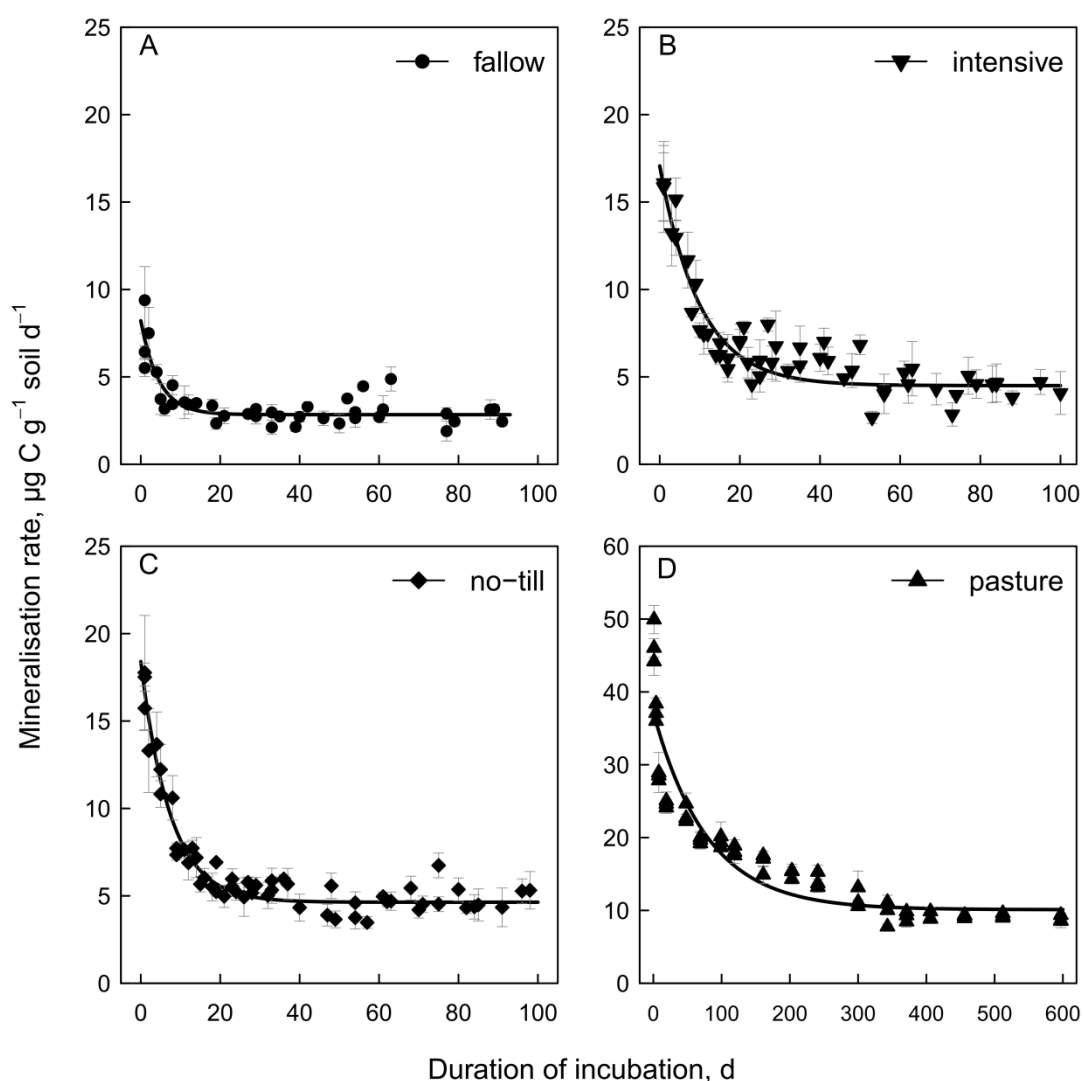


Figure 4.2. Rates of CO_2 respiration from the long-term soil incubations with an exponential decay model fitted for the (A) fallow, (B) intensive, (C) no-till, and (D) pasture treatments ($n = 3$). Data are presented as mean plot values ± 1 standard error. The best exponential decay model consisted of different equilibrium C_0 values and a and b coefficients for each treatment. Note the different scale on both axes of the pasture treatment (panel D).

4.3.4. Labile SOM estimates and effect of management

The estimates of labile SOM obtained using different measures (labile C respired, WSC, HWEC and Sand C) all showed a similar trend: the smallest values were obtained for the fallow soils and the largest for the pasture soils (**Table 4.2**). Estimates based on Sand C gave the highest proportions of labile SOM (up to 21% of SOM in pasture soils) compared with the other methods across all treatments; whereas estimates based on WSC and labile C respired were the lowest (labile C respired represented only 0.1 % of total soil C in the fallow soil, **Table 4.2**).

4.3.5. Isotopic $\delta^{13}\text{C}$ values of soil fractions

For all treatments, the equilibrium soil-respired $\delta^{13}\text{CO}_2$ values were depleted in ^{13}C compared with the solid soil, leading to negative $\Delta^{13}\text{CO}_2$ values (**Table 4.1**). The $\Delta^{13}\text{CO}_2$ values became more negative moving from the fallow soils to intensive tillage soils and even more negative values (up to -1.1 ‰) for the soils under no-till and pasture treatments.

The pattern in the $\delta^{13}\text{C}$ values of C in various fractions (sand, silt, clay, HWEC) observed across the four treatments was in agreement with the trend for the soil-respired $\delta^{13}\text{CO}_2$: respective values were significantly more depleted in ^{13}C for the pasture soil compared with the fallow soil (**Table 4.3**). A progressive enrichment in $\delta^{13}\text{C}$ with a decrease in particle-size (from sand to clay) and significant differences between the most contrasting fractions was observed for pasture soils; a similar enrichment trend was seen for the intensive and no-till soils.

Table 4.2. Soil carbon properties for the soils under fallow, intensive, no-till and pasture treatments.

Treatments	Total soil C, %	SSA ^a , m ² g ⁻¹	Labile C respired, % of total soil C	WSC ^a , % of total soil C	HWEC ^a , % of total soil C	Sand C ^b , % of total soil C	Silt C ^b , % of total soil C	Clay C ^b , % of total soil C
Fallow	1.6 ^c (0.18)a	34.7 (1.68)a	0.1 (0.01)a	0.3 (0.06)a	3.7 (0.37)a	10.6 (0.93)ab	28.7 (1.60)a	61.26 (0.21)a
Intensive	1.8 (0.07)a	28.9 (1.92)a	0.7 (0.02)a	0.6 (0.03)b	3.9 (0.13)a	14.5 (0.16)a	30.6 (0.59)a	60.73 (1.56)a
No-till	2.0 (0.03)ab	28.0 (0.98)a	0.5 (0.04)a	0.3 (0.02)a	3.5 (0.05)a	7.2 (0.25)b	17.9 (1.15)b	73.25 (1.30)b
Pasture	2.4 (0.12)b	28.8 (2.58)a	8.5 (0.80)b	1.0 (0.06)c	7.6 (0.29)b	21.0 (1.59)c	30.1 (0.53)a	46.73 (2.92)c

^aSSA – soil surface area, WSC – water soluble carbon, HWEC – hot water extractable carbon

^bSand C – sand-sized (>53 µm) carbon, Silt C – silt-sized (5 – 53 µm) carbon, Clay C – clay-sized (<5 µm) carbon

^cMean values (± 1 standard error, n = 3, soil depth 0 – 250 mm). Different lower case letters indicate significant differences between treatments based on post-hoc Tukey test (P < 0.05).

Table 4.3. Isotopic composition of solid soil carbon fractions for the fallow, intensive, no-till and pasture treatments.

Treatments	$\delta^{13}\text{C}$ HWEC ^a , ‰	$\delta^{13}\text{C}$ Sand C ^b , ‰	$\delta^{13}\text{C}$ Silt C ^b , ‰	$\delta^{13}\text{C}$ Clay C ^b , ‰
Fallow	-25.4 ^c (0.14) a A	-25.8 (0.08) a AB	-26.4 (0.08) a C	-26.1 (0.03) a BC
Intensive	-26.2 (0.09) b A	-27.0 (0.19) b B	-26.9 (0.02) b B	-26.6 (0.05) bc AB
No-till	-26.4 (0.04) b A	-26.0 (0.06) a B	-26.8 (0.08) ab C	-26.4 (0.02) b A
Pasture	-27.0 (0.13) c A	-28.0 (0.15) c B	-27.5 (0.17) c AB	-26.8 (0.13) c A

^aHWEC – hot water extractable carbon

^bSand C – sand-sized (> 53 μm) carbon, Silt C – silt-sized (5 – 53 μm) carbon, Clay C – clay-sized (< 5 μm) carbon

^cMean values (\pm 1 standard error, n = 3, soil depth 0 – 250 mm). Different lower case letters indicate significant differences between treatments (within a column), while capital letters refer to differences between measures (within a row); both are based on post-hoc Tukey test ($P < 0.05$).

4.3.6. Relationships between $^{13}\text{CO}_2$ and soil properties

Isotopic values ($\delta^{13}\text{C}$) of the HWEC fractions were the best predictor of $\Delta^{13}\text{CO}_2$ (Table 4.4). The model yielded the following equation: $\Delta^{13}\text{CO}_2 = 12.99 + 0.53 * \delta^{13}\text{C}$ HWEC; $R^2 = 0.74$; n = 12. Soil C content and proportion of labile C respired were the best traditional measures for predicting $\Delta^{13}\text{CO}_2$ values, however, these models received less support by the data ($\Delta\text{AICc} > 3.3$). A similar order of the fitted models was observed for the equilibrium soil-respired $\delta^{13}\text{CO}_2$ (Table 4.5).

Table 4.4. Summary of the models fitted to soil properties (such as hot water extractable carbon, dissolved organic carbon, labile C respired during the long-term incubations, mass proportions, C content and $\delta^{13}\text{C}$ of particle-size fractions, soil surface area and total soil C content) to explain the variability in the standardised isotopic $\Delta^{13}\text{CO}_2$ values.

Rank	Formula	d.f. ^a	AICc _i ^b	ΔAICc_i ^c	w _i ^d	cum. w. ^e
1	$\Delta^{13}\text{CO}_2 \sim \delta^{13}\text{C HWEC}$	4	12.13	0	0.55	0.55
2	$\Delta^{13}\text{CO}_2 \sim \text{Soil C}$	4	15.49	3.36	0.10	0.66
3	$\Delta^{13}\text{CO}_2 \sim \delta^{13}\text{C Clay}$	4	17.12	4.99	0.05	0.70
4	$\Delta^{13}\text{CO}_2 \sim \text{Labile C respired}$	4	17.49	5.37	0.04	0.74
5	$\Delta^{13}\text{CO}_2 \sim \text{Silt C}$	4	17.51	5.38	0.04	0.78
6	$\Delta^{13}\text{CO}_2 \sim \delta^{13}\text{C Sand C}$	4	18.12	5.99	0.03	0.81
7	$\Delta^{13}\text{CO}_2 \sim \text{Sand C}$	4	18.16	6.04	0.03	0.83
8	$\Delta^{13}\text{CO}_2 \sim \text{Clay C}$	4	18.53	6.40	0.02	0.86
9	$\Delta^{13}\text{CO}_2 \sim \text{WSC}$	4	18.55	6.42	0.02	0.88
10	$\Delta^{13}\text{CO}_2 \sim \text{HWEC}$	4	18.65	6.52	0.02	0.90
11	$\Delta^{13}\text{CO}_2 \sim \text{Sand}$	4	18.76	6.63	0.02	0.92
12	$\Delta^{13}\text{CO}_2 \sim \text{SSA}$	4	18.78	6.65	0.02	0.94
13	$\Delta^{13}\text{CO}_2 \sim \delta^{13}\text{C Silt}$	4	18.78	6.66	0.02	0.96
14	$\Delta^{13}\text{CO}_2 \sim \text{Silt}$	4	18.79	6.66	0.02	0.98
15	$\Delta^{13}\text{CO}_2 \sim \text{Clay}$	4	18.79	6.66	0.02	1

^ad.f. – degrees of freedom

^bAICc_i – Akaike's information criterion corrected for small sample sizes

^c $\Delta \text{AICc}_i = \text{AICc}_i - \text{AICc}_{\min}$, where AICc_{\min} is the model with the smallest AICc

^dw_i – Akaike weight – model probability, measure of strength of evidence of the model. This is the probability that model *i* is the KL best model, given the data and candidate set of models (Anderson, 2008)

^ecum.w. – sum of w_i of the models in a candidate set equals 1

Table 4.5. Summary of the models fitted to soil properties to explain the equilibrium soil-respired $\delta^{13}\text{C}\text{O}_2$.

Rank	Formula	d.f. ^a	AICc _i ^a	Δ AICc _i ^a	w _i ^a	cum. w. ^a
1	$y_0 \sim \delta^{13}\text{C}$ HWEC	4	15.62	0	0.44	0.44
2	$y_0 \sim \delta^{13}\text{C}$ clay	4	17.80	2.18	0.15	0.58
3	$y_0 \sim \delta^{13}\text{C}$ soil	4	17.99	2.37	0.13	0.72
4	$y_0 \sim$ Sand C	4	18.22	2.60	0.12	0.84
5	$y_0 \sim$ Labile C respired	4	19.99	4.37	0.05	0.89
6	$y_0 \sim$ Clay C	4	20.03	4.41	0.05	0.93
7	$y_0 \sim \delta^{13}\text{C}$ sand	4	21.34	5.71	0.03	0.96
8	$y_0 \sim \delta^{13}\text{C}$ silt	4	23.65	8.03	0.01	0.97
9	$y_0 \sim$ Clay	4	23.87	8.25	0.01	0.97
10	$y_0 \sim$ WSC	4	24.58	8.96	0	0.98
11	$y_0 \sim$ HWEC	4	25.00	9.38	0	0.98
12	$y_0 \sim$ Silt	4	25.12	9.50	0	0.99
13	$y_0 \sim$ Silt C	4	25.27	9.65	0	0.99
14	$y_0 \sim$ Soil C	4	25.34	9.72	0	0.99
15	$y_0 \sim$ Sand	4	25.79	10.17	0	1
16	$y_0 \sim$ SSA	4	25.80	10.18	0	1

^aAbbreviations as in Table 4.4.

The specific relationship between equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the proportion of labile C respired was analysed using the full dataset with replicate soil cores within plots. The large difference in the proportion of labile C respired between pastures and all other soils were clearly seen (**Figure 4.3**). It explained the observed treatment effect: the relationships between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the proportion of labile C respired were very different for pastures compared with all other soils (**Figure 4.3**). A strong linear relationship was obtained for the fallow and cropping soils ($\delta^{13}\text{CO}_2 = -26.30 - 1.96 * \text{Labile C respired}$, $R^2 = 0.84$, $n = 27$; **Figure 4.3**). This best-fit model with two levels of treatments (pastures and all other soils) as a fixed effect performed better than the model with four treatments, which in turn was better than the model without a treatment effect ($\Delta\text{AICc} \geq 4$, **Table 4.6**).

Table 4.6. Summary of the models fitted to equilibrium soil-respired $\delta^{13}\text{CO}_2$ as the function of labile C respired with or without Treatment as a fixed effect.

Rank	Formula	d.f.	AICc _i	ΔAICc_i	w _i	cum. w.
1	$y_0 \sim \text{Labile C respired} * \text{TR}^a$	6	23.38	0	0.91	0.91
2	$y_0 \sim \text{Labile C respired} + \text{Treatment}^b$	7	28.14	4.76	0.08	1
3	$y_0 \sim \text{Labile C respired} * \text{Treatment}$	10	35.56	12.18	0	1
4	$y_0 \sim \text{Labile C respired}$	4	41.70	18.32	0	1
5	$y_0 \sim \text{Labile C respired} + \text{TR}$	5	43.10	19.71	0	1

^aTR – treatment effect with two levels: pastures or all other soils

^bTreatment – treatment effect with four levels: fallow, intensive, no-till and pasture soils

Other abbreviations as in Table 4.4.

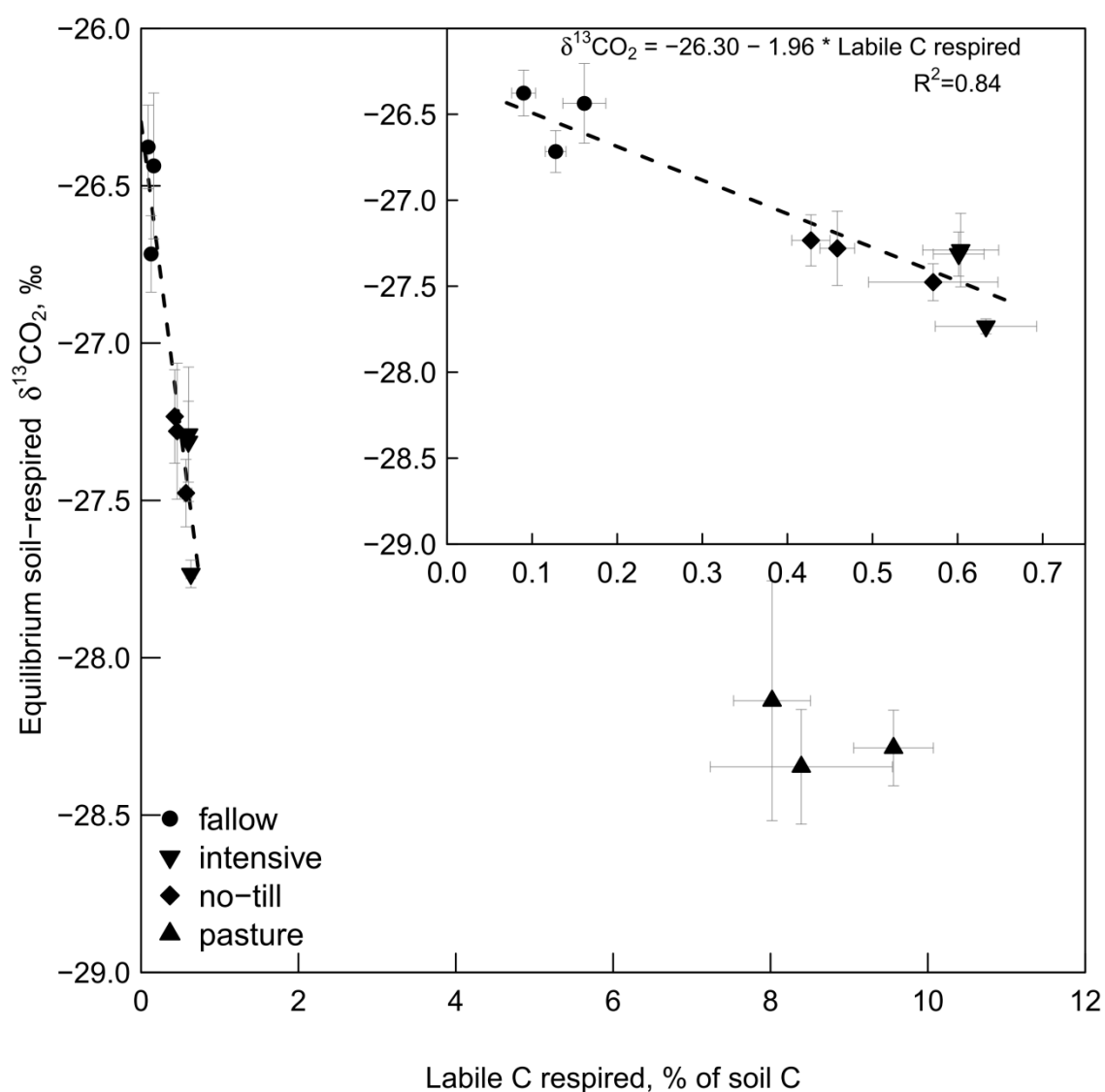


Figure 4.3. Equilibrium soil-respired $\delta^{13}\text{CO}_2$ as a function of the proportion of labile C respired for the fallow, intensive, no-till and pasture treatments ($n = 3$). Data presented as mean plot values ± 1 standard error.

4.3.7. Relationships between labile C respired and soil properties

The proportion of labile C respired, was best explained by the combination of Sand C and Silt C (**Table 4.7**). Other models received some support from the data, including the models with two variables (e.g. Sand C + Soil C). Clay C was the best single-variate model ($\Delta AICc = 3.75$, **Table 4.7**).

Table 4.7. Summary of the models fitted to soil properties to explain the proportion of labile C respired.

Rank	Formula	d.f. ^a	AICc _i	$\Delta AICc_i$	w _i	cum. w.
1	Labile C respired ~ Sand C + Silt C	5	31.05	0	0.33	0.33
2	Labile C respired ~ Sand C + SSA	5	33.41	2.36	0.10	0.43
3	Labile C respired ~ Sand C + Silt C + SSA	6	33.73	2.68	0.09	0.51
4	Labile C respired ~ Sand C + Soil C	5	33.85	2.80	0.08	0.59
5	Labile C respired ~ HWEC + Soil C + SSA	6	34.01	2.96	0.07	0.67
6	Labile C respired ~ Sand C * Silt C	6	34.20	3.15	0.07	0.73
7	Labile C respired ~ Clay C	4	34.80	3.75	0.05	0.78
8	Labile C respired ~ HWEC + Soil C	5	35.04	3.99	0.04	0.83
9	Labile C respired ~ HWEC + Soil C + Sand C	6	35.29	4.24	0.04	0.87
10	Labile C respired ~ HWEC + Sand C	5	35.54	4.50	0.03	0.90
11	Labile C respired ~ Sand C * Clay C	6	35.69	4.64	0.03	0.93
12	Labile C respired ~ Sand C	4	35.81	4.76	0.03	0.96
13	Labile C respired ~ Sand C + Soil C + SSA	6	35.81	4.76	0.03	1

^aAbbreviations as in Table 4.4.

4.4. Discussion

4.4.1. Labile SOM estimates and effect of management on labile SOM loss

Soil management regimes have demonstrated a clear influence on the amount of SOM and its labile component in particular. In this study, the soils under pasture accumulated more SOM with a larger proportion of labile SOM compared with the other treatments (**Table 4.2**). This is likely due to higher and regular plant C inputs and possibly enhanced protection of labile SOM by soil aggregates in the absence of soil disturbance (Golchin *et al.*, 1994; Six *et al.*, 2002). Soil disturbance is known to cause labile SOM depletion (Elliott, 1986; Cambardella & Elliott, 1992; Balesdent *et al.*, 2000) as regular cultivation increases macro-aggregate turnover and causes a loss of C-rich macro-aggregates and a gain of C-depleted micro-aggregates (Six *et al.*, 2000). Regular cultivation resulted in a significant reduction in labile SOM, as demonstrated by the traditional labile SOM estimates under intensive tillage compared with pastures. No-till soils were also depleted in SOM, compared with pastures, and had even less labile SOM than the soils under intensive tillage, judging by such labile SOM estimates as sand-sized C, WSC, HWE and labile C respired during long-term soil incubations. However, recently Baker *et al.* (2007) questioned the perception that conventional tillage had been the main reason of the SOM loss in the United States following intensive cultivation of the native grasslands. They suggested that the observed decrease in SOM could be a result of reduced C inputs under the annual crops compared to perennial grasses. Similarly, Sharifi *et al.* (2013) found that differences between conventional and no-till treatments were due to differences in the quantity and quality of C inputs. Moreover, Curtin *et al.* (2014) carried out a study in 2005 to test the effect of the physical disturbance and land use history on C mineralisation rates on the same tillage trial site as was used in the current study. They concluded that the decline in SOM after converting pasture to arable cropping was due to decreased amount of plant C inputs rather than increased mineralisation

rates caused by soil disturbance (such as agricultural tillage), as laboratory disturbance by sieving ($< 4\text{mm}$) did not increase mineralisation rates from the pasture soil. They have also observed that the decline in SOM was independent of the tillage intensity, which ranged from mouldboard ploughing to no tillage (Curtin *et al.*, 2014). Our results from the chemical fallow treatment are strongly in favour of this explanation. Over the 11 years without any fresh plant C inputs fallow soils became depleted in labile SOM (losing close to 50% based on sand-sized C and HWEC estimates); the remaining SOM became relatively enriched in a more recalcitrant fraction stabilised by the soil mineral matrix (indicated by a higher proportion of clay-associated C and slightly higher SSA, **Table 4.2**) Similarly, Cambardella and Elliott (1992) reported greater than 50% loss of particulate organic matter (sand-sized SOM) over 20 years of the bare-fallow management. Moreover, Barré *et al.* (2010), following soil C measurements and modelling, reported that at least 73% of the C in the Versailles soil, which has been bare-fallow for 80 years, was in a more recalcitrant, stable SOM pool.

Sand-sized SOM is quickly lost as a result of management changes and widely regarded as a labile fraction (Cambardella & Elliott, 1992; Balesdent, 1996; Christensen, 2001). The most labile SOM fraction has also been found to be relatively depleted in ^{13}C and characterised by the isotopic values close to initial plant material in a number of studies using SOM particle-size (Balesdent *et al.*, 1987; Balesdent *et al.*, 1988; Balesdent *et al.*, 1998; John *et al.*, 2005) and density fractionations (John *et al.*, 2005; Sollins *et al.*, 2006; Crow *et al.*, 2007; Dorodnikov *et al.*, 2011; Gunina & Kuzyakov, 2014; Mueller *et al.*, 2014). Labile SOM has been hypothesised to have undergone only a few biochemical changes in the preferential microbial fractionation process (Ehleringer *et al.*, 2000; Werth & Kuzyakov, 2010). According to this theory, the mineral-associated SOM is the most microbially processed and includes a larger amount of the re-synthesized SOM; thus it becomes enriched

in ^{13}C (Balesdent *et al.*, 1987; Ehleringer *et al.*, 2000; Gunina & Kuzyakov, 2014). This explains the progressive enrichment in $\delta^{13}\text{C}$ with a decrease in particle-size (from sand to clay fractions of pasture soils) observed in our study (**Table 4.3**). Moreover, pasture soils were characterised by a higher proportion of ^{13}C depleted, labile sand-sized SOM and a lower proportion of ^{13}C enriched clay-sized SOM, as well as higher traditional labile SOM estimates, compared with the other treatments. This is in good agreement with relatively ^{13}C depleted values of whole solid soil material and soil-respired CO_2 . On the contrary, fallow soils were characterised by the most enriched isotopic values and the lowest labile SOM estimates.

4.4.2. Isotopic signatures of soil-respired CO_2

As found in our previous research, the shifts in soil-respired $\delta^{13}\text{CO}_2$ over the course of soil incubations reflected changes in the labile substrate utilisation (Zakharova *et al.*, 2014). The observed rapid initial depletion of $\delta^{13}\text{CO}_2$ suggested increased availability of labile SOM, while the subsequent reversion back to the relatively enriched values indicated that labile SOM pools were exhausted (Zakharova *et al.*, 2014). Thus, short-term changes in soil-respired $\delta^{13}\text{CO}_2$ are likely to be a direct function of the amount of labile SOM and the level of its protection. A similar trend in soil-respired $\delta^{13}\text{CO}_2$ was observed with depth. ‘Topsoil’, which was likely to have more SOM, was characterised by more depleted equilibrium soil-respired $\delta^{13}\text{CO}_2$ values compared with the ‘subsoil’, across all treatments. This trend was in accordance with literature studies on $\delta^{13}\text{C}$ of SOM at different depths (Balesdent *et al.*, 1987; Huang *et al.*, 1996; John *et al.*, 2005) and was likely due to a combination of lower C inputs in deeper soil horizons as well as a higher degree of decomposition by microbes, which discriminate against the heavier C isotope (Ehleringer *et al.*, 2000; Bowling *et al.*, 2002).

In this study the pattern in equilibrium soil-respired $\delta^{13}\text{CO}_2$ and standardised $\Delta^{13}\text{CO}_2$ values across treatments were examined. Equilibrium soil-respired $\delta^{13}\text{CO}_2$ values were more

depleted in ^{13}C compared with $\delta^{13}\text{C}$ values of solid soil material (resulting in negative standardised $\Delta^{13}\text{CO}_2$ values across all treatments). This trend was in agreement with the observed by Mueller *et al.* (2014) ^{13}C depletion of the CO_2 compared with the SOM at the beginning of the incubation experiment, supporting the hypothesized microbial isotopic fractionation, with metabolic microbial ^{13}C enrichment of the SOM pool and ^{13}C depletion of the respired CO_2 (Ehleringer *et al.*, 2000). The $\Delta^{13}\text{CO}_2$ values decreased and became more negative across treatments from fallow soils to pastures. This trend relates well to the observed increase in the proportion of ^{13}C depleted, labile sand-sized SOM from fallow to pasture soils. Furthermore, this pattern can also be linked with the findings by Gunina and Kuzyakov (2014), who observed a decrease in $\Delta^{13}\text{C}$ values of the solid soil fractions with decreasing density of SOM fractions, i.e. the most labile, free light fraction was characterised with the most negative $\Delta^{13}\text{C}$ values. Gunina and Kuzyakov (2014) have linked the observed enrichment trend (from the free, most labile fraction to more stabilised, mineral fraction) with the stabilisation of SOM after passing one or more microbial utilisation cycles, with ^{13}C enrichment of microbially processed fractions (Ehleringer *et al.*, 2000; Werth & Kuzyakov, 2010). If we compare the increase in the $\Delta^{13}\text{C}$ of SOM fractions, reported by Gunina and Kuzyakov (2014), with the increase in $\Delta^{13}\text{CO}_2$ from pasture to fallow soils observed in this study (**Table 4.1**), then we can also make a link with the concept of SOM stabilisation by association with mineral particles (Six *et al.*, 2002). The fallow soils have higher $\Delta^{13}\text{CO}_2$ values, smaller proportions of labile SOM and likely more stabilised SOM due to observed slightly higher SSA with more clay-associated C. On the contrary, pasture soils are characterised by lower $\Delta^{13}\text{CO}_2$ values, a larger proportion of labile SOM, lower SSA and a smaller proportion of clay-associated C. This finding indicates that the standardised $\Delta^{13}\text{CO}_2$ values indicate the amount of labile SOM and the extent of its stabilisation in soils. Fitting models to the relationships between $\Delta^{13}\text{CO}_2$ and various traditional measures of labile SOM

showed that an alternative hypothesis ($\delta^{13}\text{C}$ of HWEC provides the best fit to the $\Delta^{13}\text{CO}_2$) was supported by the data (**Table 4.4**). HWEC is known as a sensitive measure of labile SOM (Ghani *et al.*, 2003; Haynes, 2005) and $\delta^{13}\text{C}$ of the dried powder from the hot water extractions gave the isotopic values of this labile, easily available fraction.

I was particularly interested in the relationship between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the proportion of labile C respired, as both measures are based on labile SOM respiration. The large difference in the proportions of labile C respired was, perhaps, as suggested by Curtin *et al.* (2014), due to plant C inputs: regular and substantial for the pasture soils and none for the fallow soils. I did not quantify the plant C inputs across the treatments, however, I analysed the relationships between the proportion of labile C respired and other soil properties. In this analysis, the model, which took into account both sand- and silt-associated C, provided the best-fit (**Table 4.7**). The sand-sized SOM fraction is known for its great sensitivity to disturbance and a higher potential for loss (Cambardella & Elliott, 1992). However, as found by Six *et al.* (2002), silt-associated C can also be lost. Thus, the large difference in the amount of labile C respired between the pastures and the other soils was likely due to both sand- and silt-sized C providing a source of labile SOM respiration.

Different relationships between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the proportion of labile C respired are seen for pasture and all other soils (**Figure 4.3**), with the fallow and the cropping soils showing a linear depletion in ^{13}C with the increase in labile C respired. However, the pasture soils fall out of this linear depletion trend, as if indicating plateauing in the equilibrium soil-respired $\delta^{13}\text{CO}_2$. An analogy with the soil C saturation theory (Six *et al.*, 2002; Stewart *et al.*, 2008a; Stewart *et al.*, 2008b) suggests that equilibrium soil-respired $\delta^{13}\text{CO}_2$ could be levelling out, with increasing amount of labile SOM, approaching the isotopic signature of the plant material – the source of labile SOM in soils. So, this pattern in equilibrium soil-respired $\delta^{13}\text{CO}_2$ might be similar to the C saturation curve

with its asymptote – the ultimate C saturation level (Six *et al.*, 2002; Stewart *et al.*, 2008a). A steady depletion in ^{13}C of the equilibrium soil-respired CO_2 with the increase in the amount of labile SOM up to a certain level might be similar to an increase in soil C content with increasing C inputs. This steady trend can be observed while the soil protective capacity (determined by silt + clay, micro-aggregates and biochemically protected SOM pools) is saturated. Unprotected SOM can be accumulated only above a certain soil C content and plant C input, when all protected SOM pools have been saturated. As a result of saturation, soil C content levels out, as it approaches the asymptote – the ultimate C saturation level (Six *et al.*, 2002; Stewart *et al.*, 2008a). So, I suggest that the equilibrium soil-respired $\delta^{13}\text{CO}_2$ is an indirect measure of SOM protection. A steady depletion in ^{13}C of the equilibrium soil-respired CO_2 might correspond to the saturation of the soil C protective capacity. Cropping and especially fallow soils have very little easily available, labile SOM and a higher proportion of more recalcitrant SOM (which is evidenced by a higher SSA and a larger proportion of clay-associated C). On the contrary, pasture soils have a higher proportion of easily available, labile SOM. So, as the pasture soils appear to be closer to the ultimate C saturation level compared with the other soils, perhaps the equilibrium soil-respired $\delta^{13}\text{CO}_2$ start to level out and become independent of the amount of labile SOM, as they approach the isotopic signature of the vegetation.

4.5. Summary

Soil management regimes had a clear effect on the amount of SOM and especially on the labile component (measured as WSC, HWEC, Sand C and the proportion of labile C respired). Permanent pasture soils were characterised by higher estimates of labile SOM than the soils from the other treatments. Long-term absence of fresh plant inputs in fallow soils resulted in a significant (up to 50%) depletion compared to pasture soils. The isotopic analysis of soil-respired CO_2 provides us with a tool to improve our understanding of labile SOM dynamics and its potential availability and degree of protection in soils. The equilibrium soil-respired $\delta^{13}\text{CO}_2$ became more depleted in ^{13}C from fallow to pasture soils and the standardised isotopic $\Delta^{13}\text{CO}_2$ values showed a decrease from fallow to pasture soils. These isotopic patterns were in strong agreement with the amount and availability of labile SOM across soils and were best explained by the isotopic values of the labile hot-water extractable carbon (HWEC) fraction ($\delta^{13}\text{C}$ of HWEC). Moreover, we suggest that the isotopic composition of soil-respired CO_2 reflects the degree of SOM protection in soils.

CHAPTER 5: GENERAL DISCUSSION

5.1. Summary of findings

My thesis aimed to improve understanding of the SOM dynamics, and the specific response of its labile component to soil disturbance. I was particularly motivated to use the natural abundance ^{13}C isotopic approach. This thesis has addressed the question *can the isotopic values of soil-respired CO_2 be used as a measure of the amount of labile SOM, its vulnerability and degree of protection from loss following soil disturbance?*

The following **objectives** formed the basis for this research:

1. To determine the effect of soil disturbance on labile SOM and labile C substrate utilisation by recording the shifts in soil-respired $\delta^{13}\text{CO}_2$ immediately after disturbing the soil and over the course of long-term soil incubations.
2. To determine if the short-term changes in soil-respired $\delta^{13}\text{CO}_2$ are a direct function of the amount of labile SOM and soil physical conditions. Models were fitted to identify which combination of soil properties and traditional measures of labile SOM best explained the observed equilibrium soil-respired $\delta^{13}\text{CO}_2$. Additionally, the role of soil surface area in determining labile SOM availability and protection from loss was investigated.
3. To determine if the isotopic composition of soil-respired CO_2 is a measure of labile SOM vulnerability to loss, following soil disturbance. In addition, effects of long-term regular C inputs and mechanical soil disturbance regimes on labile SOM pools, their vulnerability and protection from loss were assessed.

The main findings of this thesis are well aligned with the objectives and are summarised below:

1. Soil disturbance (due to soil sampling and breaking up the soil core) causes a rapid (within minutes of extracting a soil core) change (depletion in ^{13}C) of the isotopic signatures of soil-respired CO_2 . This trend has been observed for a range of soils (**CHAPTERS 2 and 3**) under a number of land uses (**CHAPTERS 2 and 4**).
2. This rapid isotopic change is due to labile C substrate utilisation and reflects changes in SOM protection: SOM, previously protected in intact soil, becomes available after disturbing the soil structure and determines the isotopic signatures of soil-respired CO_2 (**CHAPTER 2**). An increase in soil surface area leads to an enrichment in ^{13}C of the isotopic signatures of soil-respired CO_2 as labile SOM gets bound onto active surfaces, and thus becomes protected and unavailable (**CHAPTERS 2 and 3**).
3. Isotopic composition of soil-respired CO_2 correlates reasonably well with traditional measures of labile SOM pool (hot water extractable carbon in particular), suggesting that there is a potential for further development of the isotopic approach and converting it into a quick method of assessing the amount of labile SOM and its vulnerability to loss after soil disturbance and/or its degree of protection in soils (**CHAPTERS 3 and 4**).

The work done in this thesis has shown that the stable C isotope approach provides an effective tool to improve our understanding of SOM dynamics both in the short- and long-term. The approach registers rapid changes in isotopic signatures of soil-respired CO_2 immediately after soil sampling disturbance, on the scale of minutes, as well as slower alterations over months and years. Below, I discuss correlations with traditional labile SOM

pool estimates as well as the influence of soil disturbance on SOM in more detail and suggest the areas of further research. In addition, I link isotopic measurements with the SOM protection/saturation concept, which will enable further development of the stable C isotope approach.

5.2. Correlation with traditional measures of labile SOM pool

The work done in **CHAPTERS 3** and **4** of this thesis has shown that the isotopic composition of soil-respired CO₂ correlates reasonably well with traditional measures of labile SOM pool. In both studies hot water extractable carbon, as an estimate of labile SOM pool, demonstrated a good correlation with the equilibrium soil-respired $\delta^{13}\text{CO}_2$. Here I present the graph (**Figure 5.1**), which combines the results across different soils (**CHAPTER 3**) and different land uses (**CHAPTER 4**). While the correlation coefficient ($R^2 = 0.54$) is lower than the ones obtained in the individual studies, the overall graph has more power as it clearly shows that the relationship between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ and hot-water extractable carbon is strong regardless of differences across soils and land uses examined in this thesis. Based on these results, I suggest that with further development, the isotopic approach can be converted it into a quick method of assessing the amount of labile SOM and its vulnerability to loss after soil disturbance and/or its degree of protection in soils. I will discuss it in more detail in the section 5.5.

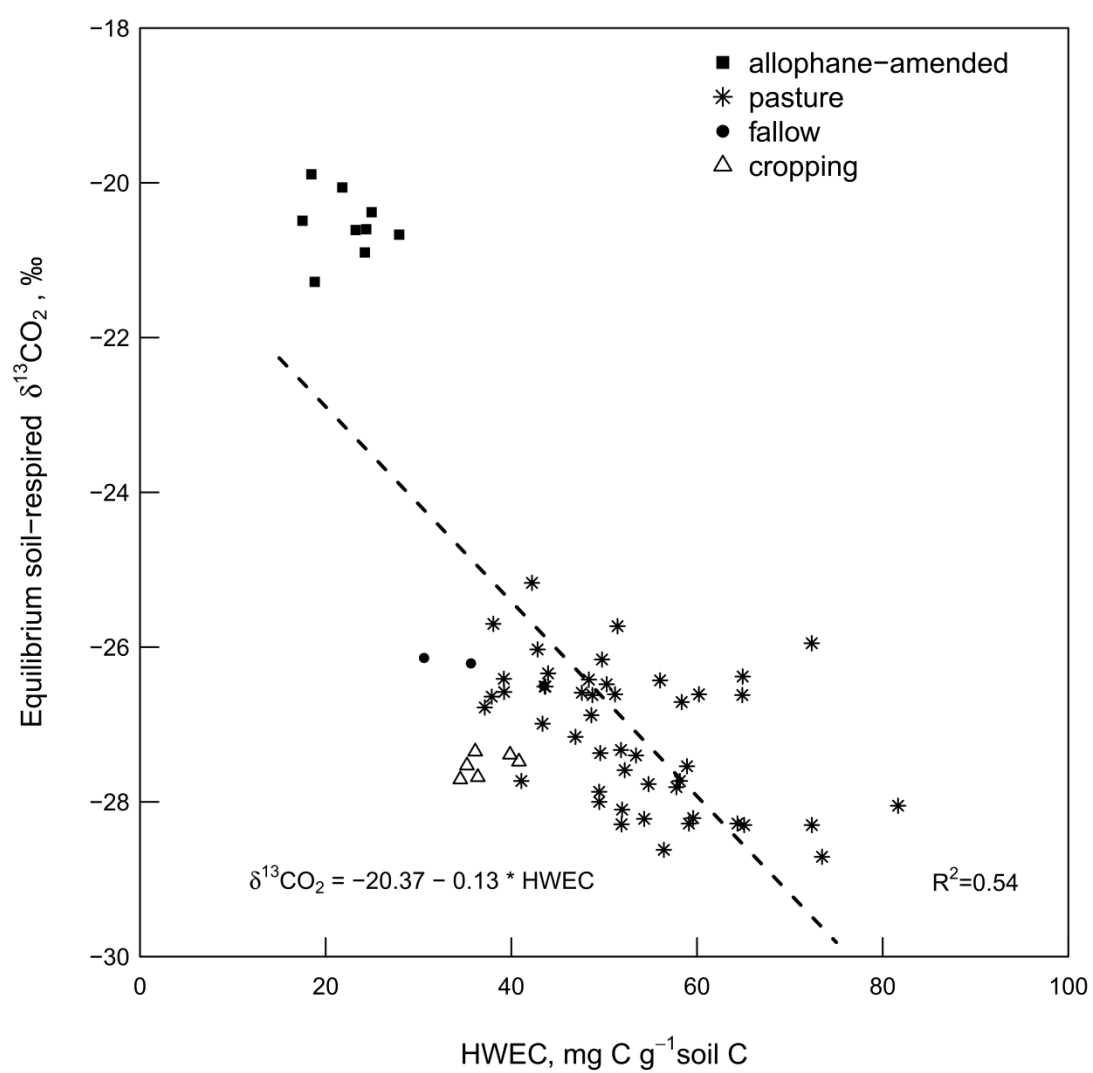


Figure 5.1. Relationship between equilibrium soil-respired $\delta^{13}\text{CO}_2$ and hot water extractable carbon (HWE) across all soils and land uses investigated in this thesis.

5.3. The influence of soil disturbance

Soil disturbance has been demonstrated to significantly affect SOM dynamics and the labile SOM pool in particular. Soil respiration rates and the isotopic signatures of soil-respired CO₂ changed immediately after the soil disturbance, suggesting that significant changes in labile SOM occur following the disturbance. This finding makes us re-assess the short-term effect of a soil disturbance. Traditional fractionation methods, which are used to separate and quantify labile SOM pools in soils (von Lützow *et al.*, 2007), usually involve a heavy soil disturbance, such as drying and sieving the soil sample through a 2-mm sieve. Physical fractionation methods include a higher disintegration by sonication to disperse soil particles. These laboratory procedures lead to a heavily perturbed state of the soil sample, which is far from its natural condition in the field. The work done in this thesis has demonstrated that labile C substrate utilisation changes after the disturbance by soil sampling. Depleted in ¹³C isotopic signatures of soil-respired CO₂ and increased soil respiration rates suggest that more labile SOM becomes available immediately after disturbing the soil, compared with the amount naturally available in intact soils. This is in agreement with the findings by Curtin *et al.* (2014), who have assessed the effect of a laboratory soil disturbance by sieving. They have concluded that soil C mineralisation increased significantly when aggregate size was below a threshold level (~ 3 mm) and it was 25 – 50% greater in fine (< 1 mm) compared with large (4 – 40 mm) particles. Moreover, heavy laboratory disturbance in its various forms destroys macro- and sometimes even micro-aggregates, which protect SOM in intact soils in the field. Thus, potentially, traditional fractionation methods could be over-estimating the amount of labile SOM available in intact soils under field conditions, as they usually include a heavy laboratory soil disturbance as a pre-treatment, which changes the degree of SOM protection and availability.

As demonstrated in **CHAPTER 2**, long-term disturbance, such as site perturbation, led to a significant reduction in the amount of labile SOM available, compared with an undisturbed soil. However, in the field it is often hard to separate the effect of soil disturbance from associated factors, such as aeration, soil temperature and moisture conditions. Moreover, long-term management history usually over-writes the effect of a laboratory soil disturbance (such as sieving), as concluded by Curtin *et al.* (2014). Similarly, as demonstrated in **CHAPTER 4**, intensity of soil disturbance (cultivation by tillage) did not significantly affect SOM loss (and labile SOM loss, in particular) after pasture cultivation. Changes in the amount and quality of plant C inputs were found to be responsible for SOM losses (Baker *et al.*, 2007; Sharifi *et al.*, 2013). So, care needs to be taken when dealing with combined influence of factors under field conditions.

In relation to the isotopic analysis, as shown in this thesis, soil disturbance causes a rapid change of the isotopic signatures of soil-respired CO₂. This disturbance effect can explain the differences between bulk soil samples and soil fractions (isotopic signatures of both solid soil material and soil-respired CO₂). It also explains the observation that the sum of the isotopic signatures of soil fractions does not necessarily equal the isotopic value of the whole soil sample, as shown in **CHAPTER 4** of this thesis and literature studies (Balesdent *et al.*, 1987; Balesdent *et al.*, 1988; Balesdent *et al.*, 1998; John *et al.*, 2005; Dorodnikov *et al.*, 2011).

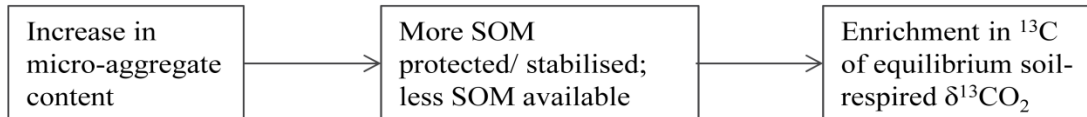
5.4. Linking isotopic signatures with conceptual SOM pools

The concept of SOM protection against soil disturbance can also help link the isotopic measurements with various SOM pools and SOM saturation theory (Six *et al.*, 2002; Stewart *et al.*, 2008a; Stewart *et al.*, 2008b). According to the theory, accumulation of SOM and the ultimate soil C saturation level in soils are determined by physicochemical characteristics of the four SOM pools: labile unprotected, physically protected by micro-aggregates, chemically protected by silt and clay particles, and biochemically protected (**Figure 1.3A** in **CHAPTER 1**). **Figure 5.2** suggests links between the changes in the SOM pools and equilibrium soil-respired $\delta^{13}\text{CO}_2$, either based on the findings of this thesis or literature overview. An increase in labile unprotected SOM pool leads to depletion in ^{13}C of equilibrium soil-respired $\delta^{13}\text{CO}_2$. However, an increase in any of the protected SOM pools (or change of soil conditions leading to this increase) will result in enrichment in ^{13}C of equilibrium soil-respired $\delta^{13}\text{CO}_2$ (**Figure 5.2**).

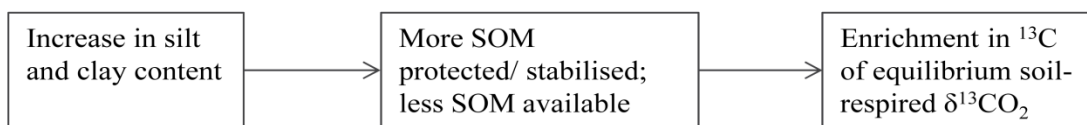
1. Labile unprotected SOM pool



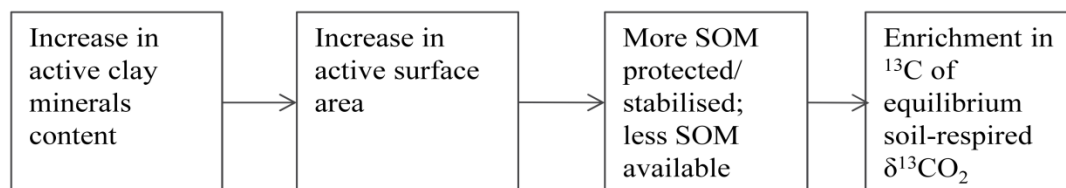
2. Micro-aggregate protected SOM



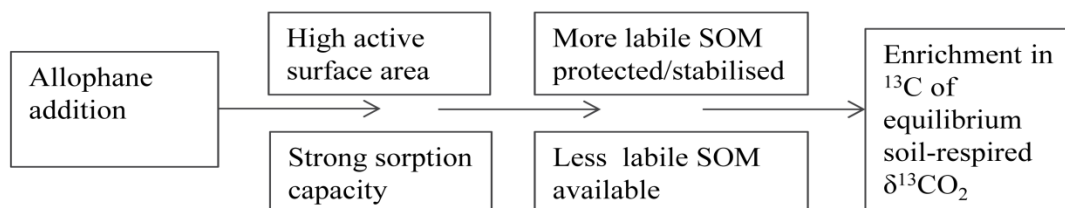
3. Silt and clay protected SOM



Influence of soil mineralogy



Allophane addition



4. Biochemically protected SOM

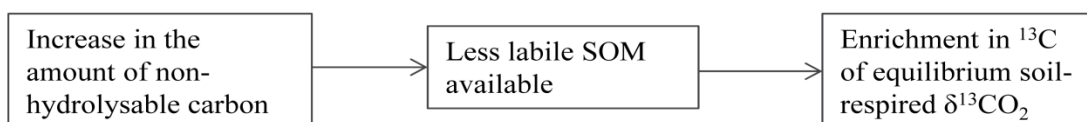


Figure 5.2. Linking isotopic signatures of soil-respired CO₂ with the conceptual SOM pools from the SOM saturation theory, developed by Six *et al.* (2002).

5.5. Further development of the approach

5.5.1. Clarifying the labile SOM vulnerability curve

As suggested in the thesis, the isotopic signatures of soil-respired CO₂ indicate SOM availability and, perhaps, the degree of its protection/vulnerability to loss following soil disturbance. In **CHAPTER 2** of this thesis I carried out long-term soil incubations and concluded that the shifts in soil-respired $\delta^{13}\text{CO}_2$ over the course of incubations reflected changes in the labile substrate C utilisation. Further studies, coupling long-term soil incubations with measurements of soil-respired $\delta^{13}\text{CO}_2$ and soil C mineralisation rates, will allow directly testing the hypothesis that soil-respired $\delta^{13}\text{CO}_2$ indicates the amount of labile SOM and its vulnerability to loss in soils. Both measures – isotopic composition of soil-respired CO₂ and soil C mineralisation rates – are based on the labile SOM respiration. Thus, a simultaneous plateauing of soil C mineralisation rates and a reversion of soil-respired $\delta^{13}\text{CO}_2$ back to the starting, more enriched values, will prove that the trends in soil-respired $\delta^{13}\text{CO}_2$ indicate the amount of labile (vulnerable to loss) SOM in soils. Furthermore, they will allow robust testing of the isotopic method to assess labile SOM vulnerability to loss and quantify potential losses.

In **CHAPTER 4** I carried out long-term soil incubations and measured soil C mineralisation rates and analysed the relationship between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the proportion of labile C respired (**Figure 4.3**). As a result, I suggested that the equilibrium soil-respired $\delta^{13}\text{CO}_2$ is an indirect measure of SOM protection. However, I am not certain how this relationship between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the proportion of labile C respired will look, given the wider range of the amount of labile SOM, total soil C content and C inputs. It might be similar to the relationship between plant C inputs and soil C content on the C saturation curve (Six *et al.*, 2002). A steady depletion in ^{13}C of the equilibrium soil-respired CO₂ with an increase in the amount of labile SOM up to a

certain level is similar to an increase in soil C content with increasing C inputs. This steady trend is observed while silt + clay, micro-aggregate and biochemically protected SOM pools are saturated up to the soil protection level. Unprotected SOM is accumulated only above this soil protection level with a certain soil C content and plant C input. When all the SOM pools have been saturated, soil C content levels out and approaches the asymptote – the ultimate C saturation level (Six *et al.*, 2002; Stewart *et al.*, 2008a). So, ideally, a number of study sites on the same soil type with a wider range of total soil C content (possibly 1 – 8%) are needed to clarify the relationship between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ and the proportion of labile C respired. This will allow verifying the shape of the labile SOM vulnerability curve on a wider range of total soil C content.

5.5.2. Linking with soil C protection capacity

In **CHAPTER 4** I suggested that equilibrium soil-respired $\delta^{13}\text{CO}_2$ is a measure of soil C protection capacity. Moreover, **Figure 5.2** links isotopic signatures of soil-respired CO_2 with soil C protection and saturation theory. So, further research in this area will directly test the hypothesis that equilibrium soil-respired $\delta^{13}\text{CO}_2$ is a measure of soil C protection capacity. The studies should couple soil incubations and isotopic measurements with quantification of all the SOM pools from the C saturation theory, not just labile SOM pools. Isotopic measurements provide a quick tool for recording the response of the SOM as a whole, without artificially separating it into pools based on the methodological protocols and assumptions. Thus, correlations between the isotopic measurements and estimates of various discrete SOM pools will provide valuable information on how different SOM pools contribute to a bigger picture of SOM as a complex structure. Additionally, we will be able to say if, using the isotopes, it is possible to measure various discrete pools obtained using fractionation methods, and how they are protected in the soil. A range of soil C content and C inputs will provide a wider range of SOM pools, especially the labile, unprotected one.

Carrying out a similar study on a number of contrasting soil types will also be very beneficial. Using contrasting soil types (e.g., allophanic vs non-allophanic) will serve as a good link to the allophane addition experiments (**CHAPTER 3**) and provide a great comparison as the soil protection level will be higher in allophanic soils (mainly due to the textural and mineralogical composition). It has been shown that at the same mass proportion of fine soil particles (clay and fine silt), soils dominated by 2:1 minerals have more C associated with fine particles compared with the soils with 1:1 minerals (Hassink *et al.*, 1997; Feng *et al.*, 2013). Beare *et al.* (2014) compared New Zealand pasture soils and demonstrated the same trend for allophanic vs non-allophanic soils. So, allophanic soils are characterised by a higher total soil C content as more SOM is protected by silt and clay, micro-aggregates and biochemically. However, given a higher soil C protective capacity and protection levels, a higher total soil C content is required before unprotected SOM starts to accumulate. This means that, at the same level of total soil C content, the isotopic composition of the soil-respired CO₂ is likely to be more enriched in ¹³C for the allophanic soils compared with non-allophanic soils. This hypothesis is based on the results of the allophane addition experiment (**CHAPTER 3**), where the isotopic signatures became more enriched in ¹³C and the amount of labile SOM decreased when the SSA increased. It would also be interesting to quantify which of the protected SOM pools has the largest influence on the change in δ¹³CO₂. In order to do so, we need to find two sites, one on the allophanic soil and the other on a non-allophanic one, with a similar total soil C content and similar soil texture (sand, silt and clay content). However, I would expect that the size of the protected pools in the allophanic soil will be higher due to mineralogical composition (mainly higher capacity of allophane and other clay minerals to absorb and stabilise SOM). By quantifying clay, silt, micro-aggregate (<250 µm) and biochemically (non-hydrolysable fraction) protected SOM pools in both soils and correlating them with equilibrium soil-respired δ¹³CO₂ we will be able to identify, which

of the pools contributes the most to the isotopic responses of the soils. In addition, it would be interesting to analyse the relationships between the soil-respired $\delta^{13}\text{CO}_2$ and total soil C content (not just the proportion of labile SOM), and potentially get a response similar to the C saturation curve. As to the comparison between allophanic and non-allophanic soils, I would expect that at the same soil C content, the equilibrium soil-respired $\delta^{13}\text{CO}_2$ will be more enriched in ^{13}C in the allophanic soils, compared to non-allophanic ones.

5.5.3. Cross-calibrating with the radio-carbon approach

As mentioned in **CHAPTER 1** of this thesis, the radiocarbon approach has been widely used to quantify the turnover of SOM pools on a timescale of years to decades. So, another avenue for further research is comparison and possible cross-calibration of the stable C isotope approach with the radiocarbon method. Bearing in mind that the bomb ^{14}C method might not be very reliable with the short-term SOM response (Trumbore, 2000; 2006), I suggest to start the comparison with longer time scales, perhaps, my long-term incubations could be a good starting point (**CHAPTERS 2 and 4**). However, I am really interested in correlation between the equilibrium soil-respired $\delta^{13}\text{CO}_2$ values, which indicate short-term SOM dynamics and availability, and $\Delta^{14}\text{CO}_2$ values, obtained within the same timeframe. Another idea worth pursuing would be to undertake a study on a soil chronosequence. A river valley might be a good site for this research as soils will be of different age. Higher fluvial terraces are remnants of earlier floodplains when the river was flowing at a higher elevation before its channel downcut (as a result of erosion processes) to create a new floodplain at a lower elevation. As a result, the fluvial terraces will be older compared with the current floodplain. Another possibility is the Franz Josef chronosequence in the central western South Island, New Zealand, where repeated glacial advance and retreat has created a series of schist outwash surfaces dating from more than 120,000 years ago to the present (Richardson *et al.*, 2004). Isotopic analysis of soil-respired $\delta^{13}\text{CO}_2$ will characterise short-term SOM

dynamics and availability across all soils, whereas $\Delta^{14}\text{CO}_2$ will likely provide an estimate of a medium to long-term SOM turnover. Moreover, the radiocarbon method may be able to determine the age of soils and estimate SOM dynamics in the long-term (up to thousands of years). I suggest that correlations between all isotopic measurements ($\delta^{13}\text{CO}_2$, $\Delta^{14}\text{CO}_2$, $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$) across all the scales will enable a cross-calibration of the approaches on a wider timescale. This further development of the natural abundance ^{13}C approach may allow us to use this cheaper (compared with bomb ^{14}C) method on a variety of scales to quantify SOM availability and dynamics.

I would like to conclude the section about further development of the natural abundance ^{13}C approach by quoting from the review by Kuzyakov (2006). The fact that this approach “does not require any special equipment for plant labelling and isolation from the atmosphere combined with the recent developments in spectroscopic methods to measure $\delta^{13}\text{CO}_2$ (infrared tunable diode laser absorption spectroscopy, Fourier Transform infrared spectroscopy and near-infrared spectroscopy), which are cheaper to install/maintain and do not require special sample preparation or separation of gases (as in common mass spectrometry) *will promote the use of ^{13}C natural abundance approach in future investigations*”.

5.6. Conclusions

The natural abundance ^{13}C approach provides us with a tool to investigate SOM dynamics, both on shorter and longer time scales. Isotopic analysis of soil-respired $\delta^{13}\text{CO}_2$, in particular, enables the recording of changes immediately after soil disturbance and linking them with the changes in the labile SOM pool. The shifts in soil-respired $\delta^{13}\text{CO}_2$ over the course of soil incubations were found to be due to changes in labile C substrate utilisation. The observed rapid initial depletion of $\delta^{13}\text{CO}_2$ immediately after disturbing the soil suggests increased availability of labile SOM; while the subsequent reversion back to the relatively enriched values over a period of several months indicates that labile SOM pools were exhausted. Moreover, further research identified that the shifts in soil-respired $\delta^{13}\text{CO}_2$ were a direct function of the amount of labile SOM and soil physical conditions. In particular, equilibrium soil-respired $\delta^{13}\text{CO}_2$ indicated not only the amount of labile SOM, but also its availability in soils, mainly determined by soil surface area. Higher soil surface area resulted in a decrease in hot water extractable carbon (as an estimate of the labile SOM) and a relative enrichment in ^{13}C of the equilibrium soil-respired $\delta^{13}\text{CO}_2$ as demonstrated by incubating soil with allophane. Furthermore, the isotopic patterns were in strong agreement with the amount and availability of labile SOM across a number of management treatments and were best explained by the isotopic values of the labile hot-water extractable carbon fraction ($\delta^{13}\text{C}$ of HWEC). As a result, I suggest that the isotopic composition of soil-respired CO_2 reflects the degree of SOM availability and protection in soils. However, further studies are needed to develop the soil-respired $\delta^{13}\text{CO}_2$ approach into a quick method of assessing labile SOM availability, its vulnerability to loss and degree of protection in soils.

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APPENDIX #1

Loss of labile carbon following soil disturbance determined by measurement of respired $\delta^{13}\text{C}\text{O}_2$

CrossMark

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ARTICLE INFO

Article history:

Received 30 April 2013

Received in revised form

28 September 2013

Accepted 2 October 2013

Available online 14 October 2013

Keywords:

Carbon isotope discrimination

Labile soil organic matter

Soil respiration

Incubation

Hot water extractable carbon

Allophane

ABSTRACT

Soils are the largest pool of carbon (C) in terrestrial ecosystems with labile C being particularly vulnerable to loss. In this study we incubated a range of soils in both the short- (minutes) and long-term (months) to assess the loss of labile soil C by measuring the isotopic signature of soil respired CO_2 ($\delta^{13}\text{C}\text{O}_2$). Strong temporal trends in $\delta^{13}\text{C}\text{O}_2$ values were observed following soil disturbance: $\delta^{13}\text{C}\text{O}_2$ rapidly changed from a range of -22.5 to -23.9 ‰, to -25.8 to -27.5 ‰, during short-term incubations and reverted back to the initial values in long-term incubations. The shifts in $\delta^{13}\text{C}\text{O}_2$ over the course of soil incubations were consistent with changes in labile C substrate utilization following the disturbance of sampling the soil. An independent experimental approach which immobilised labile soil C onto allophane and included chemical extractions as a measure of extractable C in soils also confirmed this interpretation. Collectively, these results indicate that the isotopic analysis of respired CO_2 can be a powerful technique which enables us to probe mechanisms and examine the consequences of disturbance on the labile component of soil C.

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<http://dx.doi.org/10.1016/j.soilbio.2013.10.001>

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